A STUDY ON URINARY BACTERIAL AND FUNGAL ISOLATES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN FROM PATIENTS UNDERGOING INVASIVE UROLOGICAL OP DIAGNOSTIC PROCEDURES INCLUDING CYSTOSCOPY AND URODYNAMIC STUDY

Dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

In partial fulfillment of the regulations

for the award of the degree of

M.D.(MICROBIOLOGY)

BRANCH – IV

MADRAS MEDICAL COLLEGE

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

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CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON URINARY BACTERIAL AND FUNGAL ISOLATES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN FROM PATIENTS UNDERGOING INVASIVE UROLOGICAL OP DIAGNOSTIC PROCEDURES INCLUDING CYSTOSCOPY AND URODYNAMIC STUDY"** is a bonafide record work done by Dr.M. Vijii, during the period of her Post Graduate study from MAY 2012 to APRIL 2015 under guidance and supervision in the 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 2015.

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DECLARATION

I declare that the dissertation entitled **"A STUDY ON URINARY BACTERIAL AND FUNGAL ISOLATES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN FROM PATIENTS UNDERGOING INVASIVE UROLOGICAL OP DIAGNOSTIC PROCEDURES INCLUDING CYSTOSCOPY AND URODYNAMIC STUDY"**submitted by me for the degree of M.D. is the record work carried out by me during the period of October**2013 –** September**2014** under the guidance of **Dr.G.Jayalakshmi, M.D.,D.T.C.D.,** Director & Professor of Microbiology, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical Unversity, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Branch IV (Microbiology) examination to be held in April 2015.

Place : Chennai
Date:

Signature of the candidate
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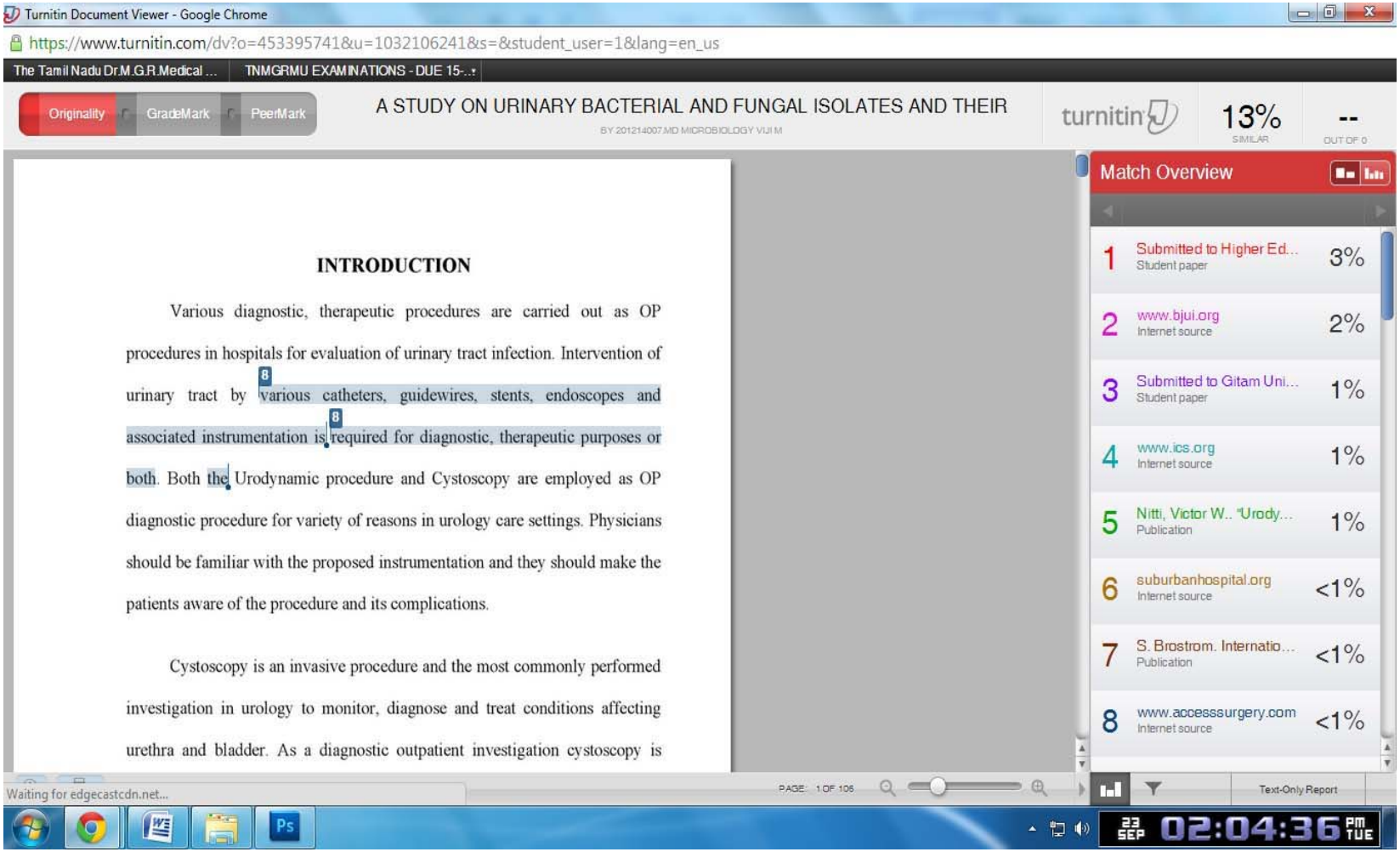
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INTRODUCTION
Various diagnostic, therapeutic procedures are carried out as OP procedures in hospitals for evaluation of urinary tract infection. Intervention of urinary tract by various catheters, guidewires, stents, endoscopes and associated instrumentation is required for diagnostic, therapeutic purposes or both. Both the Urodynamic procedure and Cystoscopy are employed as OP diagnostic procedure for variety of reasons in urology care settings. Physicians should be familiar with the proposed instrumentation and they should make the patients aware of the procedure and its complications.
Cystoscopy is an invasive procedure and the most commonly performed investigation in urology to monitor, diagnose and treat conditions affecting urethra and bladder. As a diagnostic outpatient investigation cystoscopy is
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CERTIFICATE OF APPROVAL

To

Dr. Viji . M.,
Post Graduate in MD Microbiology,
Institute of Microbiology,
Madras Medical College, Chennai-3.

Dear **Dr. Viji . M.,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled ***"A study on urinary Bacterial and fungal isolates and their antimicrobial susceptibility pattern from patients undergoing invasive urological OP diagnostic procedures including Cystoscopy and Urodynamic study "*** No.16122013

The following members of Ethics Committee were present in the meeting held on 11.12.2013 conducted at Madras Medical College, Chennai-3.

1. Dr. G. Sivakumar, MS FICS FAIS	-- Chairperson
2. Prof. B. Kalaiselvi, MD Vice Principal, MMC, Ch-3	-- Member Secretary
3. Prof. Ramadevi, Director i/c, Instt. of Biochemistry, Chennai.	-- Member
4. Prof. P. Karkuzhali, MD for Dr. V. Ramamoorthy Prof. Instt. of Pathology, MMC, Ch-3	-- Member
5. Thiru. S. Govindasamy, BABL	-- Lawyer
6. Tmt. Arnold Saulina, MA MSW	-- Social Scientist

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

Sd/Chairman & Other Members

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

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
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CONTENTS

A STUDY ON URINARY BACTERIAL AND FUNGAL ISOLATES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN FROM PATIENTS UNDERGOING INVASIVE UROLOGICAL OP DIAGNOSTIC PROCEDURES INCLUDING CYSTOSCOPY AND URODYNAMIC STUDY

ABSTRACT

Objectives. To isolate and identify the bacterial and fungal causative agents for post procedure of urinary tract infection in patients undergoing cystoscopyand urodynamic (UD) evaluation in a prospective survey. To study the association of ompT virulent gene for E.coli with significant colony counts. The tolerability and acceptance of and UD studies by patients have not been thoroughly documented. It would be helpful to be able to give such information to patients before performing these procedures.

Methods. A total of 200 patients seen as outpatients for Cystoscopy and Urodynamic studies to evaluate various indications were studied. A midstream urine sample was taken before,third day and seventh after the procedures and semiquantitative culture was employed for colony counts. Detection of virulent gene ompT for *E.coli* by conventional PCR. Patients were given a questionnaire that inquired about the presence of lower urinary tract symptoms after the procedures.

Results. Of the 200 patients analyzed (Cystoscopy100, UD studies 100), 9 patients (4.5%) developed significant bacteriuria .*E.coli* (40.9%) was the commonest isolated organism.ompT gene was negative for *E.coli* with significant colony counts. Only 2 patients(2%) with significant bacteriuria symptoms . The association between patients who had symptoms with significant growth and underlying condition after the procedure (n = 2) was significant ($P<0.05$). ompT gene

Conclusions. Cystoscopy and Urodynamic studies are safe, well-tolerated procedures. The antibiotics for the transieint infection of these procedures is unnecessary, unless specific indications are present.

Key words- ompT-outer membrane protein, PCR-polymerase chain reaction

INTRODUCTION

Various diagnostic, therapeutic procedures are carried out as OP procedures in hospitals for evaluation of urinary tract infection. Intervention of urinary tract by various catheters, guidewires, stents, endoscopes and associated instrumentation is required for diagnostic, therapeutic purposes or both. Both the Urodynamic procedure and Cystoscopy are employed as OP diagnostic procedure for variety of reasons in urology care settings. Physicians should be familiar with the proposed instrumentation and they should make the patients aware of the procedure and its complications.

Cystoscopy is an invasive procedure and the most commonly performed investigation in urology to monitor, diagnose and treat conditions affecting urethra and bladder. As a diagnostic outpatient investigation cystoscopy is incorporated firmly and widely performed for various urological diseases such as haematuria, symptoms associated with lower urinary tract and surveillance of bladder cancer following resection of tumour and radiation therapy.

Though traditionally considered as a clean procedure it is associated with marked morbidity [4] and risk of complications including bleeding, infection and pain. Despite the relative low rate of UTI there remain a number of patients requiring parenteral antibiotics for complicated UTI. And also it has the risk of outbreaks of infection due to organisms including *Pseudomonas aeruginosa*, secondary to scope contamination and processing failures [5].

Urodynamics is the dynamic study of the storage, transport and evacuation of the urinary tract. This procedure is performed for disorders of lower urinary tract mainly in patients with incontinence and forms an important part of evaluation of neuropathic disorders in neuro urology and in female geriatric patients involving urinary tract. Selecting a patient for surgery based on history, physical examination, endoscopy or radiographic findings would be inappropriate. So further altered physiology of urinary tract must be investigated by urodynamic tests to devise an appropriate surgery for the patients.

Catheters are employed for urodynamic evaluation. It is a mild invasive procedure. Catheters are the tools that destroy the host immune system and let microorganisms to enter normally under aseptic conditions [6, 7]. Adequate lubrication is very important than sterility before insertion of catheter. Urodynamic studies are associated with an increased risk of bacteriuria and symptomatic urinary tract infection (UTI) because it is applied by catheterization [9].

There is no thorough documentation of acceptance and tolerability of these two procedures by the patients. Cystoscopy and Urodynamic study has a risk of infection and this should be taken into account. It should be included in counselling for the patients undergoing these procedures as a part of good

clinical practice. Patients were requested to collect midstream urine [MSU] sample to the microbiology laboratory at the time of procedure , third day and seventh after the procedure.

Infection is the recognized complication of instrumentation in the lower urinary tract [1]. Infection of one or more structures in the urinary system is called as UTI. The most common site of nosocomial infections is the urinary tract. Catheterization and instrumentation are the major predisposing factors for UTI [2].Wide range of UTIs have been reported after lower urinary tract invasive procedures [1]. Invasive urological procedures account for up to 10 % of the nosocomial infections [3].

Active screening for UTI before these two procedures to avoid inappropriate intervention based on false-positive study [12]. Urine samples were collected under strict aseptic precautions and processed without delay. Defining the CFU/ml that represents clinically significant infection can be difficult. Colony counts were determined by semi-quantitative culture of the urine to determine whether it contains a potentially pathogenic bacteria in numbers sufficient to identify it as the causal infecting organism. Significance of the colony counts were interpreted in relation to the clinical information of the patient [10].

Aims and Objectives of the study

AIMS AND OBJECTIVES

1. To isolate and identify the bacterial & fungal causative agents for post invasive procedure of urinary tract infection in patients undergoing Cystoscopy and Urodynamic procedure.

2. To perform semi-quantitative count and to define the urinary tract infection.

3. To study the association of significant bacteriuria and candiduria separately withCystoscopy and Urodynamic procedure.

4. To study the antibacterial &antifungal susceptibility pattern for the isolates.

5. To study the association of virulent gene (ompT) in *E.coli* with significant colony counts as it is commonest isolate in causing urinary tract infection after post cystoscopy and urodynamic study.

6. To compare and evaluate relative merits of each of these invasive procedures as OP urological investigation procedure.

Review of literature

REVIEW OF LITERATURE

Manipulation of urinary tract by various instruments such as urethroscopes, rectoscopes, cystoscopes and various catheters were used in urology care setting for various therapeutic, diagnostic purposes or both. These instruments were used to manipulate the urinary tract in retrograde manner without the need to open through surgery. This feature differentiates urology and other clinical fields. Care should be taken while applying these instruments over the patients since it can cause significant injury while manipulating the urinary tract. Physician should understand the procedure, its limitations and sterile precautions which includes preparing patients before the procedure, sterile irrigating solutions and sterilizing instruments usedinbetween the patients.

NORMAL FLORA

Many micro-organisms are located in the urethra and vulva, and contaminate the urine in its passage, so that normally freshly voided urine is never sterile, but always contain a certain number of bacteria, some of them simple and harmless and others of them are dangerous and pathogenic. These organisms find their way into the bladder from the urethra, usually by the passage of instruments, or by creeping along the short and wide urethra of the female [14].

Anaerobic bacteria, *Lactobacilli,* C*orynebacteria, Streptococci* (not including *Enterococci*), and *Staphylococcusepidermidis* are found in normal periurethral flora. They do not commonly cause UTIs in healthy individuals and are considered common urinary contaminants [14].

PATHOGENESIS [13]

ANTIBACTERIAL HOST DEFENCES IN THE URINARY TRACT

Antibacterial substances such as flushing action of urine, urinary pH, presence of organic acids, bactericidal activity of urine, inhibitors of adherence of bacteria present in the host protects from bacterial infection. While inserting the catheter, or any instruments involving retrograde manipulation of urinary tract for diagnostic, therapeutic purposes or both it may carry the organisms present in the urethra into the bladder and cause infection even after following strict aseptic precaution. The incidence of bacteriuria ranging from about 1% and 20 % in healthy and elderly hospitalized patients respectively. [16,17].

Among the three pathways the ascending, hematogenous, and lymphatic pathways ascending pathway of infection is the most important. UTI is more common in women than in men gives support to the importance of the ascending route of infection. Contamination is more in females than males since the female urethra is short and proximal in position to the vulvar and

perineal areas. Colonization of organisms takes place in women before urinary infection results.

UROVIRULENCE IN BACTERIA

Among the urinary pathogens *E.coli* is the most commonest uropathogen particularly O, K, and H serogroups. These particular serogroups are capable of adhering to uroepithelium by means of P pili and type 1 pili. Once attachment to uroepithelial cells occurs, other bacterial pathogenic factors such as hemolysin, which initiates tissue invasion and makes iron available for the infecting pathogens and cause infection of urinary tract. the presence of K antigen on the invading bacteria protects them from phagocytosis. The K antigen present in the pathogens allow the infecting pathogens to escape the various host defenses.

In addition to above factors there are many virulent genes associated with uropathoenic strains. They are ompT , CNF1, adhesins, iha and hlya. ompT gene is a house keeping gene protease present in most outer membrane of Gram negative bacteria that degrades foreign peptide in the surrounding environment and it is associated with pathogenesis in urinary tract infection, intestinal colonization and sepsis.

CAUSATIVE PATHOGENS

E.coli causes80 % of the uncomplicated urinary tract infection (Orskov et al). *Klebsiellaspp, Proteus spp, Enterobacter spp*. *Pseudomonasspp and Staphylococcus spp*and*Enterococci* are other uropathogens causing hospital – acquired UTIs (Wagnenlehner and Naber, 2000). *Staphylococcus saprophyticus*, once often thought as urinary contaminants, can cause uncomplicated UTI in young women. (Hovelius and Mardh, 1984).

EPIDEMIOLOGY OF URINARY TRACT INFECTION

More than 95 % of UTIs are caused by a single bacterial species. In acute infection*E.coli* is the most common causeofinfection[19, 20]. Infection caused by *Proteus, Pseudomonas, Klebsiella, Enterococci spp*and *Staphylococci* increases greatly in patients with structural abnormalities because of instrumentation and repeated courses of antimicrobial therapy.

Fungi particularly candida species occur in patients with indwelling catheter. The prevalence of bacteriuria in young women is about 1% to 3% and adult men is 1%[21]is lowcompared to 10 % in older men and 20% in older women. During their life time of women experience 40 to 50% of symptomatic UTI[22, 23]. Neuro muscular diseases, increased instrumentation and bladder catheter usage in both genders [24] are possible reasons in older patients .

URODYNAMIC STUDY

HISTORY [26,27]

Davis in the year 1954 coined the term urodynamics though instruments for bladder pressure measurement and rate urine flow has been developed in the year 1800s itself(Davis 1954; Perez and Webster 1992).

Measuring the various pressures is the most important part of urodynamic study.The year 1950 is the infant period of modern urodynamics.VonGarrelts' and Davis published work on measurements of uroflowmetry and cystometry pressures simultaneously inspired a lot (Perez and Webster 1992).Von Garretin the year 1956 and in 1963Zinner and Paquinreported the normal micturition pressures electronically in men and in women (Zinner and Paquin 1963) respectively.

Following them in 1960 reintroduction ofmeasurement of micturition pressures by Murphy and Schoenberg and in 1962 combination use ofcystometry and uroflowmetryby Gleason and Lattimerfor bladder outlet strictures was called as the pressure-flow study (Gleason and Lattimer1962).The achievement of these basic researchdrew back the prologue of modern urodynamic studies[28].

Urodynamic studies provides valuable information regarding etiology and act in concert with clinical history and physical examination of the patient for appropriate intervention.

INDICATIONS

1. To identify or to rule out the factors contributing to the incontinence and their relative importance
2. To obtain information about other aspects of the lower urinary tract dysfunction
3. To predict the consequences of the dysfunction for the upper urinary tract
4. To predict the outcome and undesirable side effects of a contemplated treatment.
5. To confirm the effects of treatment or to understand the mode of action of a particular type of treatment, especially a new one.
6. To understand the reasons for failure of previous treatments for incontinence

SAFETY

Screening of urine for bacteriuria at the time of the testes important to rule out unrecognized infection. Antibiotics administered at or just after the study, are at thediscretion of the investigator. The main risks of cystometry are those associated with urethral catheterization. Dysuria (painful voiding) occurs

in some patients after urodynamic testing, but usually disappears within 48 hour. The technique used for catheterization, and for handling of transducers and connecting tubes, varies in different centers from clean to sterile. It is not known whether these variations have any effect on the infection rates. In any case, appropriate aseptic techniques should be used.

URODYNAMIC PROCEDURE

TECHNIQUE

Patients were asked to attend for their investigations with a full urinary bladder. The urinary flow rate was initially measured and the patients were encouraged to empty their bladder completely.The vulva and external urethral meatus were washed with sterile aqueous solution containing chlorhexidine gluconate 0.015% w/v and cetrimide 0.15% w/v. After adequate lubrication with a sterile lubricating gel, the bladder pressure catheter (4.5 F) was inserted above and alongside the bladder-filling catheter (10 F).

The post void residual volume (PVR) was then collected by catheterization, and a catheter specimen of urine obtained at this time and sent for bacteriological assessment. Both catheters were taped in place, care being taken to avoid blockage of the bladder pressure catheter.

A rectal balloon catheter (4.5 F) with a perforated latex sheath (to avoid fecal plugging) was similarly introduced into the rectum to measure the intra-

abdominal pressure. The bladder-filling catheter was then connected to the filling tube and pump. The bladder and rectal pressure catheters were connected to the bladder and rectal external transducers, respectively, by proximal extension tubes filled with sterile normal saline. These proximal tubes were regularly replaced between patients to minimize the chances of cross-infection.

Urodynamic studies were carried out according to standard criteria [33] in the erect position with an Albyn Medical (Griffon, UK) urodynamic unit and a multichannel recorder. The bladder was filled at 100 mL/min with sterile normal saline at room temperature until either 500 mL had been infused, or unstable detrusor activity or patient discomfort precluded further filling.

On completing the filling cystometry, all catheters were withdrawn and the uroflowmetry repeated. Patients were usually encouraged to void to completion, after which the PVR was assessed by simple subtraction (voided volume from infused volume) and if thought significant, evaluated further with ultrasonography.

Disinfection of catheters

Cidex (2% glutaraldehyde)

It is a high-level disinfectant for heat-sensitive reusable medical devices that cannot undergo sterilization. Most commonly used disinfectant for

medical devices.Catheters used in the urodynamic procedure were disinfected by 2% glutraldehyde solution for 30 minutes before starting the procedure, in between the patients and air dried before storage.

RISK ASSOCIATED WITH URODYNAMIC TESTING

SIDE EFFECTS

- discomfort in the area where a catheter was put in – this get better after a few hours
- mild stinging when passing urine
- blood in urine
- Urinary tract infection infection

CYSTOSCOPY

HISTORY [34]

The cystoscope which is used nowadays is the outcome of many centuries of development.In the year 1805 Bozinni's introduced the protype of urethral viewing tube. These protypesweredifficult to use for the operator, caused patient inconvenience and it is dangerous for both.

The images were inverted and backwardsby early models and needed the skill of operator to correct this image. Objects appeared small and distant since it is illuminated by reflected candlelight. With the advanced Nitze's prototype, a forerunner of the current cystoscope which utilizes lenses, prisms

and the incandescent light image quality was improved.Cystoscopehas broadened the scope of urology since its introduction.

Image quality,educational opportunities and quality of patient care was unparralled with the invention of fibre optics and digital imaging unimaginableto the early pioneers.

Cystoscopy can assist in identifying problems with the urinary tract, such as early signs of cancer, infection, strictures (narrowing), obstruction, and bleeding. Cystoscopy is an endoscopic technique for examining the internal aspect of the bladder. It is the principle way to diagnose and surveil bladder conditions.

Indications to perform cystoscopy

Cystoscopy can be used for diagnostic and operative indications. It is being used in gynecologic and non-gynecologic conditions [35,36]

- Identifying intraoperative injuries gynecologic malignancies involving urinary tract
- Genitourinary fistulas
- Traumatic involvement of the urinary tract
- Irritative voiding symptoms in the absence of urinary tract infection (urinary urgency or frequency or urge incontinence)
- Urethral diverticulum
- Verification of suprapubic catheter placement

Indications to perform cystoscopy for non-gynecologic conditions:

- Recurrent urinary tract infections
- Hematuria
- Urinary incontinence or overactive bladder
- Abnormal urine cytology
- Chronic pelvic pain, interstitial cystitis and painful urination
- Urinary blockage caused by stricture, or narrowing of the urinary tract
- Stone, unusual growth, polyp, tumor, or cancer in the urinary tract

Types of Cystoscopy

Based on flexibility and type of anaesthesiaused there are two main types of cystoscopy - flexible and rigid . In flexible cystoscopy, the diameter is usually 15 to 18 French. The fiber optic telescope and irrigation channel are combined in a single unit.[37]

Differences between rigid and flexible cystoscope

Use of flexible fiberopticcystoscopes is associated with less pain and postoperative morbidity than rigid cystoscopies [38]. However, the flow rate of irrigation fluid is less than with rigid cystoscope and visualization is not as clear. Use of a flexible cystoscope requires more extensive training than a rigid scope. Use of flexible cystoscopes where no anesthesia, topical anesthetic or

conscious sedation is used and rigid cystoscopes where regional or general anesthesia is used.

Procedure

It can be performed either in OP set up or in operating room. Patient is positioned in dorsal lithotomy position and areas weresteriely prepared.Cystoscope — A rigid cystoscope consists of a telescope, bridge connector, sheath, and obturator (fits inside the sheath) [37]is inserted into the urethra and advanced in to the blader.A 30 or 70 degree telescope is usually used for cystoscopy, whereas 0 degree lens is preferred for urethroscopy. The 0 and 12 degrees lenses focuses largely straight ahead and are excellent to evaluate the urethra, but often times are inadequate for visualization of the entire bladder [39, 40].

The 30-degree lens allows optimal visualization of the posterior wall and the base of the bladder. The 70-degree lens allows visualization of the dome, anterolateral walls, and into an elevated urethrovesical junction, such as after colposuspension procedures. Finally, the 120-degree retro-lens allows the visualization of the bladder and the urethra, and optimizes visualization of the bladder neck [39, 40].

The sheath contains channels for instilling irrigation fluid and inserting operative instruments. The diameter of the sheath that is used commonly is 17

to 24 French. It is advised to use the smallest cystoscope that allows good visualization and to minimize pain and trauma to urethra. In selected cases, such as urethral compression by a tumor, using a pediatric cystoscope (8 French) may be helpful [41]. At times, even with the smallest scope, Urethral dilatation is necessary. Gradual dilatation using Hegar dilators may be done. [39, 40].

Telescope cables, which are either fibroptic or fluid-filled, serve as the illuminating system [39, 40].even though the fluid-filled cables tend to last longer, the fibroptic cables are being used more commonly because they are less expensive.

Distending medium

Irrigating fluid is instilled to distend the bladder and improve visualization. There are different distending media conductive fluids (laclated ringer's, normal saline), nonconductive or non-electrolyte (sterile water, 5% glycine, 3% sorbitol, 5% mannitol), and gas. The usual distending medium for most surgeons for diagnostic procedures is isotonic saline or sterile water, because of better visualization.

Use of any distending medium requires monitoring of fluid absorption to avoid volume overload. Use of nonconductive fluids (eg, glycine) may result

in hyponatremia, and therefore, these fluids are reserved for operative procedures.

Examination of the lower urinary tract using cystoscope

Urethra —The urethral mucosa must be examined for pallor, exudates, polyps, erythema, condylomata, or diverticulae[37].

Bladder —The dome of the bladder can often be identified by the presence of an air bubble. Slight suprapubic pressure can facilitate visualization of the dome.

A bubble-like appearance to the trigone, or bullous edema, may indicate tumor encroaching upon the bladder, but does not document bladder invasion for staging purposes. Frond-like papillary growths or shaggy, necrotic tissue may be a tumor (either primary or extending into the bladder) and should be biopsied. A biopsy is performed with a cystoscopic biopsy instrument. After the biopsy, the biopsy site usually has no bleeding or minimal bleeding that does not require cautery.

Ureteral orifices — the trigone is directly in front of the urethra and is identified by locating the interureteric ridge. The cystoscope may need to be rotated to visualize the ureteral orifices. Several minutes of observation may be needed to identify the orifices, which are small, slit-like openings that only become apparent when a burst of urine is released. Use of any distending

medium requires monitoring of fluid absorption to avoid volume overload. It is important to mention that hyponatremia can happen following use of nonconductive irrigation solution.

Reasons for the Procedure

A cystoscopy may be recommended when a disorder of the urinary tract is suspected. Urinary tract disorders may include structural problems that can lead to a blockage of urine flow or a back flow of urine. If untreated, structural problems may lead to potentially serious complications. Cystoscopy may also be performed after gynecologic surgical procedures near the bladder to check for proper placement of sutures and support devices.

Sterility of instruments

Cidex (2% glutaraldehyde)

It is a high-level disinfectant for heat-sensitive reusable medical devices that cannot undergo sterilization. Most commonly used disinfectant for medical devices. Cystoscopes require a systematic process to be reusable which includesprecleaning, leak testing, cleaning, disinfection with 2% glutraldehyde solution (Cidex) for 30 minutes, rinsing, and drying before allowing for storage**.**

Risks of the Procedure

- Bleeding
- Retention of urine
- Perforation of bladder
- Infection

LABORATORY DIAGNOSIS [42]

SPECIMEN COLLECTION

For the laboratory diagnosis of UTI proper collection of urine sample is essential. An important consideration of a clinically relevant urine specimen is without the contamination of normal perineal, vaginal and anterior urethral flora. For the collection of urine sample different methods have been employed which are as follows;

CLEAN CATCH MIDSTREAM URINE

The most common technique for the collection of urine samples is the midstream flow by the clean catch technique. The perineum and periurethral area was first cleaned with soapy water followed by the rinse with the sterile saline or water.

WOMEN

During voidingthe labia should be held apart. To flush out bacteria from the urethrathe first few millilitres of urine is passed and then midstream urineis collected in a sterile wide mouthed container with tightly covered with lid.

MEN

Immediately before voidingcleanising of the urethral meatus and then midstream urine is collected is usually sufficient [43] .

STRAIGHT CATHETERIZED URINE

Employed for the patients who are unable to void urine sample by midstream clean catch technique. By this method bladder urine is collected with reduced urethral contamination by discarding few millimetres of urine coming out from the catheter. Catheterization for this purpose is no longer considered justifiable .The main drawback of this method is that it carries risk of introducing and initiating infection.

SUPRAPUBIC ASPIRATION

With strict aseptic precautions the procedure is done when the bladder is full. This method of urine sample collection is reserved for children and neonates. By this method contamination of the distal urethra is avoided.

SPECIMEN TRANSPORT

Delay in the transport of urine specimen to the laboratory causes the contaminating bacteria to multiply readily since urine is an excellent culture medium. If there is unavoidable delay of 1-2 hours urine sample can be stored in a refrigerator at 4°C or by addition of 1.8% to the urine sample can prevent bacterial mutltiplication.

Processing of urine samples in lab

MICROSCOPY OF URINE

WET FILM EXAMINATION [42]

It is useful in detecting pus cells, bacteria, red blood cells, casts or crystals, yeast and parasites in urine.

GRAM STAINING OF URINE SPECIMEN [42]

Finding of one bacterium per oil immersion field correlates with 10^5 bacteria or more per ml of urine. Absence of bacteria in several fields correlates with fewer than 10^4 bacteria per ml [44] .

CHEMICAL METHODS

Leucocyte esterase test

This test detects enzyme leucocyte esterase present in intact as well as lysed leucocytes. In the presence of vaginal fluid, eosinophils and *Trichomonasvaginalis* false positive results are possible.

Greiss test

Enterobactericeae group of bacteria produce nitrite from nitrate. Greiss test detects the presence of nitrite in urine. The strip is impregnated with test reagent . Since*S. saprophyticus, Enterococci, Pseudomonas* species do not produce nitrite usefulness of this test is limited. The first produced urine sample is required since the bacteria requires more than four hours to convert nitrate in to nitrite.

Simultaneous use of these two tests results in better performance. But Griess test and leukocyte esterase have low sensitivity, high specificity, high negative predictive values and low positive predictive values [45]

AUTOMATED AND SEMI AUTOMATED SYSTEMS [46]

Several semi-automated and automated systems for urine screening systems are commercially available. Uncentrifuged urine samples can be examined through video camera Sysmex UF -100 and IRIS 939 UDX system. These systems are designed to recognize bacteria and leucocytes. A robotic instrument cellenium 160- US utilises fluorescent probes to stain bacterial monolayer from urine on a membrane and under computerized fluorescent microscopy imaging technology it is examined at high magnification. Limited clinical data have been published. A variety of automated urinary screen systems are in developmental stages and been widely accepted. [45]

SIGNIFICANT BACTERIURIA [42]

Although the greater part of urinary tract is devoid of commensal flora and bladder urine in an uninfected person is free from bacteria, a specimen of spontaneous voided urine is apt to be contaminated with some commensal bacteria from the urethral orifice and perineum, particularly in females, even when the most careful precautions are taken to prevent such contamination.The concept of significant bacteriuria was first introduced by Kass (1956). He demonstrated that the presence of more than 10^5 cfu/ml in a single specimen of urine indicated significant bacteriuria with a probability of >80 % and when 2 or 3 consecutive specimens are examined it could be increased to >90 % or up to 99% when 2 or 3 consecutive specimens are examined .[47]

Rigid adherence to Kass's concept should be avoided. Counts between 10^3 -10^5 are significant in certain clinical situations. Decreased counts are obtained when increased fluid intake dilute the urine in symptomatic patients or they may have received antimicrobial therapy in the recent past. The bacterial count in some patients with uroepithelial damage and recurrent infection may not reflect clinical realities [48].

ASYPTOMATIC BACTERIURIA

Significant bacteriuria may sometimes occur in the absence of symptoms and pyuria in patients who subsequently develops symptoms of urinary tract infection, eg. Pregnancy. The detection of such asymptomatic bacteriuria is of value, for there is good evidence of its association with the development of pyelonephritis in some patients.[49]

FUNGURIA

Mostly caused by candida and has become increasingly prominent in hospitals. Candida albicans was the single most common microbial species isolated. [50] Candiduria is presence of yeast cells in urine. Most patients with candiduria are asymptomatic. Colony count of $>10^5$ /ml of urine is associated with infection in patient without indwelling catheters. Clinically significant renal candidiasis has been reported even with low colony counts of 10^3 /ml of urine. Candiduria most likely reflects colonization or infection of lower urinary tractor collecting system of kidneys.[51]

SEMI- QUANTITATIVE CULTURE METHODS [42]

Though it is ideal to count the viable bacteriuria in urine by Miles and Misra method or pour plate method, they are laborious to use. So standard loop method or filter paper method is used.

STANDARD LOOP METHOD [42]

A Nichrome or Platinum wire of SWG 28 is used. It is made into circular loop of 3.26 mm internal diameter. It will hold a drop of water or urine of 0.004 ml. When loops holding 0.004 ml or more are used and spread uniformly over whole of the plate, colonies are so numerous as to be confluent and ther is no single, separate colonies available for picking and the preparation of pure subcultures. Loops with fixed volume of 0.001 – 0.01 ml are used for semi- quantitative culture. One standard loopful of urine is delivered and spread over CLED or over Sheep blood agar plate. Incubate the plates aerobically at 35 – 37 ° C overnight and number of colonies grown counted and expressed as CFU/ml of urine multiplying count by 1000 if 0.001 ml of loop is used or 100 if 0.01 ml of loop is used. [42]

FILTER PAPER METHOD [42]

Bent a standard 6mm wide absorbent filter paper strip into L shape with 12 mm long foot. Sterilize the strip by hot air oven at 160° C for 1 hour. Whole strip is dipped in the urine, drain the excess fluid and place the foot of the strip over an agar plate medium. Remove the strip and incubate the plate at 35-37^{o}C overnight.

Colonies growing on the plate are counted. 25 colonies of bacilli or 30 colonies of cocci corresponds 10^{5} CFU/ml approximately.

DIP SLIDE METHOD

It is a small plastic tray containing a layer of agar culture medium. Different media on both sides of the tray. The dip slide is briefly immersed in the midstream urine collected in a sterile container, drain the excess urine and the disk is replaced in its container. After incubated at 37 ° C overnight and it is examined for growth. The number of colonies grown or pattern of growth whether semi-confluent or confluent is compared with charts from the supplier to determine the viable count of bacteria [42]

IDENTIFICATION AND SENSITIVITY OF THE TEST

Similar colonies found in numbers suggesting significant bacteriuria and candiduria are identified by the apppearances of the primary growth on CLED or Mac Conkey medium.10^2 CFU ml is also significant in symptomatic patients. Colony counts between 10^4 and 10^5 or when multiple species are recovered , decision depends on clinical information of the patient. Antibiotic sensitivity depend upon the isolate and clinical condition of the patient [42]. As antibiotics are concentrated in urine to higher levels than are found in the tissues, high content discs should be used. [49]

Materials and methods

MATERIALS AND METHODS

Study period

This cross sectional study was carried out in the Institute of Microbiology, Madras Medical College in association with Department of Urology, Rajiv Gandhi Government General Hospital, Chennai over a period of one year. This study was reviewed and approved by Institutional ethical committee, Madras Medical College.

Patient's undergoing Urodynamic procedure and Cystoscopy were taken under the study. Informed consent was obtained from the study population. All patients satisfying the inclusion criteria were documented. Patients were interviewed by structured questionnaire. All data were handled anonymously and confidentially.

SAMPLE COLLECTION

They were requested to give three samples of midstream urine 'clean catch' to the microbiological laboratory in a wide mouthed universal container with a secured lid. A proper instruction was given to the patient regarding the collection of midstream urine sample.

Male patients were asked to retract the prepuce, cleanse the glans penis, with soap and water and then collect the sample from the middle urine flow.

Female patients were instructed thoroughly to clean the ano-genital area from front to back, pass urine with labia separated and collect sample from middle portion of stream.

Three samples of urine were collected from the patients. First urine sample was collected just before the procedure, second urine sample was collected at the third day and third urine sample was collected at the 7^{th} day after the procedure.

SAMPLE SIZE

PROCEDURE	NUMBER OF PATIENTS	NUMBER OF SAMPLES
Cystoscopy	100	3X100=300
Urodynamic study	100	3X100=300
TOTAL	200	600

Inclusion criteria

1. Patients who were selected for cystoscopy and urodynamic study.
2. Patients who were above 18 years of age.
3. Samples which were found to be negative for urine culture prior to the procedure and on the day of the procedure.

Exclusion criteria:

1. Samples which were found to be positive for urine culture prior to the procedure and on the day of the procedure.
2. Patients on antibiotics.

STUDY:

SAMPLE PROCESSING

Under strict aseptic precautions samples were collected from the patients and since urine is an excellent culture medium supporting rapid growth of many bacteria it was transported immediately to the laboratory in appropriate settings and sample processing was done within one hour.

WET FILM EXAMINATON [42]

Urine sample was mixed carefully and about 0.05 ml of urine was placed in the middle of the microscopic slide. At once a NO.1 cover slip of 22x22 mm in dimensions was placed over it, taking care to avoid air bubbles. The preparation was placed under high power objective [40x] of light microscope. The number of pus cells per high power field was recorded. Observation was also done for the presence of epithelial cells, red blood cells, parasites, yeasts (budding yeast cells and pseudohyphae) and bacteria. All these findings were recorded.

GRAM STAINING [42]

A drop of well mixed uncentrifuged urine was air dried over a microscopic slide, heat fixed and Gram's staining was carried out. The stained smear was examined under oil immersion [100x] for bacteria and yeast cells and their number per field was recorded.

TABLE-3 BACTERIAL COUNT BY DIRECT EXAMINATION OF URINE [44]

SAMPLE	UNSTAINED CFU/ml [wet mount]	STAINED CFU/ml [Gram stain]
UNCENTRIFUGED	$\geq 10^6$	$\geq 10^5$
CENTRIFUGED	$\geq 10^5$	$\geq 10^4$

CFU/ml –extrapolated from the finding of one bacterium per microscopic field.

CFU/ml-colony forming units.

SEMI QUANTITATIVE CULTURE [42]

A calibrated loop that delivers 0.001 ml of urine was used to culture urine sample semi-quantitatively. A loopful of urine was surface plated on CLED agar. Urine sample was mixed thoroughly, the calibrated loop was inserted vertically in to the urine sample and the sample was inoculated on CLED media and streaked to obtain individual colonies.

Streaking technique[52]

Nonferrous (Nichrome or platinum) or disposable plastic inoculating loops, calibrated to contain 0.001 ml of fluid, was immersed into an uncentrifuged urine sample. The loop was then carefully removed and the entire volume was delivered to the surface of an agar plate by making a single streak across the center. The inoculum was spread evenly at right angles to the primary streak: then the plate was turned 90 degrees and the inoculum was spread to cover the entire surface.

The plated cultures were incubated at 37° C for 24 hours. The number of colonies grown on the surface of the agar were counted after 18 to 24 hours of incubation and interpreted as CFU/ml of urine by multiplying the number of colonies grown by 1000. The colony counts exceeding 10^5 CFU/ml was taken as significant colony count.

IDENTIFICATION AND SENSITIVITY TEST

Colonies suggesting bacteriuria / candiduria were processed. Phenotypic identification of uropathogens was carried out by means of biochemical reactions. Selection of battery of test depended on organism.

ISOLATION, IDENTIFICATION AND SPECIATION OF BACTERIAL ISOLATES

Growth in CLED medium was identified. Lactose fermenting [LF] colonies were identified based on motility, catalase test, nitrate reduction, methyl red test, VogesProskauer, production of indole, H_2S, urease, citrate utilization and sugar fermentation tests. Non lactose fermenting [NLF] colonies were grouped under *Enterobactericeae* based on oxidase production, catalase production, reduction of nitrate to nitrite.

Further speciation depended on motility, fermentative/oxidative metabolism of sugars, phenyl alanine deaminase test and battery of sugars, production of indole, H_2S, urease, citrate utilization and decarboxylation of lysine, arginine and ornithine in Moeller's decarboxylation media.

Gram positive cocci morphologically resembling Staphylococci were identified by coagulase test and novobiocin sensitivity. Suspected enterococci colonies were identified by bile esculin agar, growth in the presence of 6.5 % sodium chloride and fermentation of sugars in peptone water sugar media.

DETECTION OF MOTILITY [42]

Motility was done by hanging drop method for actively motile bacteria. Detection of motility is important in identifying the bacilli.

Antimicrobial sensitivity tests[42]

Antimicrobial sensitivity tests was carried out by Kirby Bauer disc diffusion method on Mueller Hinton agar [Hi Media, Mumbai, India].The media was prepared by suspending 38 grams of dehydrated media in 1000 ml distilled water, autoclaved at 121 ° C for 15 minutes and poured in to petri dishes to a depth of 4 mm. A broth culture of the isolate with turbidity equivalent to Mc Farland 0.5 turbidity standard was lawn cultured over the Mueller Hinton agar and allowed to dry.

The antibiotic discs contained in the catridges were taken out from the refrigerator half hour earlier and brought to room temperature. Antibiotic discs were applied to MH agar surface seeded with test bacterium and incubated at 37 ° C. The antibiotic discs used were from Hi Media and the following antibiotic discs were used amikacin, ampicillin, Penicillin, Amoxyclave, Cephalexin, Ceftazidime, Cefotaxime, Ciprofloxacin , Gentamicin, nitrofurantoin, norfloxacin ,Erythromycin, Cefoxitin, High level gentamycin and Tetracycline.

The antibiotic susceptibility test was interpreted as sensitive or resistant by comparing the inhibitory zone size produced by test organism with that of standard strains of bacteria. The standard strains used were *Staphylococcus aureus* ATCC 25923 ,*Escherichia coli* ATCC 25922 and *Pseudomonas*

aeruginosa ATCC 27853.The selection of battery of antibiotic discs was based on organisms isolated from UTI.

ISOLATION, IDENTIFICATION AND SPECIATION OF COMMON FUNGAL ISOLATES

Colonies resembling candida were identified by growth at 37 ° C, Gram's stain and germ tube test. Further speciation of candida by cornmeal agar, Chrom agar, sugar fermentation and assimilation tests.

Gram stain

From an isolated colony a smear was made and stained by Gram's stain and examined under oil immersion for the presence of gram positive budding yeast cells and pseudohyphae.

Germ tube test

An isolated colony of yeast was added to 0.5 ml of human serum in a sterile test tube and incubated at 37°C for two hours. A drop of this suspension is placed on a slide with cover slip applied over it and observed for presence of germ tube under high power microscope.

Positive - *Candida albicans / Candida dubliniensis*

Negative - *Candida non albicanspp*

CORN MEAL AGAR [Dalmau plate culture technique]

Using a straight wire an isolated colony from primary culture was inoculated by making three parallel lines half an inch apart into corn meal agar plate and covering the portion of the streaks with sterile coverslip. Examined under low power and then under high power objective after 48 hours of incubation at 25˚C where cuts are made for the presence of hyphae, blastoconidia, arthroconidia and chlamydospores.

TABLE-5 MORPHOLOGY ON CORN MEAL AGAR

Candida albicans	Terminal chlamydospores.
Candida tropicalis	Abundant pseudohyphae, pine forest arrangement, blastoconidia formed at or in between septa.
Candida krusei	Elongated yeasts, abundant pseudohyphae(match stick like appearance)
Candida guilliermondi	Giant hyphae, blastospores at nodes.
Candida glabrata	Only yeasts.
Candida lusitaniae	Short, distinctly curved pseudohyphae with occasional blastoconidia at septa.
Candida parapsilosis	Fine pseudomycelium with single or small clusters of blastoconidia and giant cells.
Candida kefyr	Abundant pseudomycelium of elongate cells that lie parallel giving log in stream appearance. Infrequent blastoconidia.

CANDIDA CHROM AGAR

A single yeast colony from sub culture of Sabourauds Dextrose agar was streaked on to plates. After incubation of 48 hours at 37°C the colour and the colony morphology was noted.

TABLE-6 APPEARANCE ON CHROM AGAR

SPECIES	COLOUR	MORPHOLOGY
Candida albicans	Green	-
Candida tropicalis	Steel blue	-
Candida krusei	Pink pale borders	Medium to large flat, rough.
Candida guilliermondi	Pink to lavender	-
Candida glabarata	Dark violet	Small to medium, smooth convex creamy.
Candida lusitaniae	Pink to lavendar	Waxy, large rough
Candida parapsilosis	Ivory to pink to lavender	Small to medium, smooth wrinkled.
Candida kefyr	Pink to lavender	Large, rough

SUGAR FERMENTATION TESTS

0.2 ml suspension of 24-48 hours culture from sugar free media was added to 2% sugar fermentation media with Andrade indicator and incubated at 30 ° C for 48 to 72 hours. Acid and gas production indicates fermentation. Glucose, maltose, sucrose, lactose sugars were tested.

TABLE-7 FERMENTATION REACTION

SPECIES	GLUCOSE	MALTOSE	SUCROSE	LACTOSE
C. albicans	F	F	-	-
C. tropicalis	F	F	F/V	-
C. krusei	F	-	-	-
C.guilliermondi	F	-	-	-
C. glabrata	F	-	-	-
C. lusitaniae	F	F	-	-
C. parapsilosis	F	-	-	-
C. kefyr	F	-	F	F

F- acid and gas, V- variable

SUGAR ASSIMILATION TEST

To the yeast suspension from 24- 48 hours culture grown in sugar free media 2 ml of yeast nitrogen base was added. To this 18 ml of molten agar added, cooled to 45° C, poured into sterile petri plate and allowed to set. Carbohydrate discs [Hi Media] dextrose, sucrose, lactose, trehalose and cellibiose were placed on the agar and incubated at 30 ° C for 24-48 hours. Growth around the discs indicates assimilation.

TABLE-8 CARBOHYDRATE ASSIMILATION

SPECIES	GLUCOSE	TREHALOSE	SUCROSE	LACTOSE	CELLEBIOSE
C. albicans	+	+	v	-	-
C. tropicalis	+	+	V	-	+
C. krusei	+	-	-	-	-
C.guilliermondi	+	+	+	-	+
C. glabrata	+	+	-	-	-
C. lusitaniae	+	+	+	-	+
C. parapsilosis	+	+	+	-	-
C. kefyr	+	-	+	+	+

+ positive, - negative, V variable

ANTIMICROBIAL SENSITIVITY TESTING

ANTIBACTERIAL SUSCEPTIBILITY

Disc diffusion method :

Antibiotic sensitivity was assessed by Kirby –Bauer disc diffusion method using the Mueller-Hinton agar plate for all the isolates.

The isolate was sub cultured on sheep blood agar, to obtain a pure culture. To get the organism in the logarithmic phase three to four colonies were suspended in nutrient broth and were incubated for two hours at 37ºC. The density of the suspension was standardized to 0.5 McFarland units. Within fifteen minutes of preparation of the suspension, a sterile cotton-wool swab was dipped into the suspension and the surplus was removed by rotating the swab against the side of the test tube.

To obtain a lawn culture the agar plate was inoculated by even stroking of the swab over the entire surface of the plate in three directions. The antibiotic discs were placed, six on each plate after drying. With each batch of tests, a control for each antibiotic was also set up. As per the CLSI guidelines the control strains were included and obtained from American Type Culture Collection (ATCC).

Following panel of drugs were used for antimicrobial sensitivity testing

TABLE-9 Drugs used for antimicrobial sensitivity testing

ANIBIOTICS	DISC CONTENT	RESISTANT (mm)	INTER-MEDIATE (mm)	SENSITIVE (mm)
Cefotaxime *Enterobactericeae,* *Acinetobacterspp*	30µg	≤22 ≤14	23-25 15-22	≥26 ≥23
Ceftazidime *Enterobactericeae,* *Acinetobacterspp*	30µg	≤14 ≤18	18-20 15-17	≥ 21 ≥18
Amikacin *Enterobactericeae* *S.aureus,* *Pseudomonasaeruginosa,* *Acinetobacterspp*	30µg	≤ 14	15-16	≥ 17
Gentamycin *Enterobactericeae,* *Pseudomonas* *aeruginosa,* *Acinetobacterspp*	10µg	≤ 12	13-14	≥ 15
Ciprofloxacin *Enterobactericeae,* *S.aureus, Pseudomonas* *aeruginosa,* *Acinetobacterspp*	5µg	≤ 15	16-20	≥ 21
Ampicillin *Enterobactericeae* *Enterococcus spp*	10µg	≤13 ≤8	14-16 -	≥17 ≥17
Nitrofurantoin *Enterobactericeae, S.* *aureus, Enterococcus spp*	300µg	≤14	15-16	≥17
Norfloxacin *Enterobactericeae,* *S.aureus, Enterococcus* *spp, Pseudomonas* *aeruginosa*	10µg	≤12	13-16	≥17

ANIBIOTICS	DISC CONTENT	RESISTANT (mm)	INTER-MEDIATE (mm)	SENSITIVE (mm)
Cefoxitin *S.aureus,*	30µg	≤21	-	≥22
Amoxyclav Enterobactericeae	20/10µg	≤ 13	14-17	≥ 18
Tetracycline *Enterobactericeae* *S.aureus*	30µg	≤ 11 ≤14	12-14 15-18	≥ 14 ≥19
Penicillin *S.aureus* *Enterococcus spp*	10units	≤28 ≤14	- -	≥29 ≥14
High level gentamicin *Enterococccusspp*	120µg	6	7-9	≥10
Trimethoprim-sulfamethoxazole *S.aureus,* *acinetobacterspp*	1.25/23.75µg	≤10	11-15	≥16
Erythromycin *S.aureus, Enterococcus spp*	15µg	≤13	14-22	≥23

ESBL Detection by Disc Potentiation Test:

Jariler et al. first described this method in 1988 for Enterobacteriaceae. In this method a lawn culture of test isolate was made as per CLSI guidelines.[48]Ceftazidimeclavulanic acid disc and ceftazidime disc was placed at a distance of 30mm from centre to centre. The test isolate was considered to produce ESBL, if the zone size around the β lactamase inhibitor combination disc was increased by ≥5mm. ATCC Escherichia coli 35218 was used as control.

Minimum inhibitory concentration (MIC) for Vancomycin by macrobroth dilution method

1. Culture media:

Cation adjusted Mueller Hinton broth (pH 7.2-7.4)

ANTIBIOTIC STOCK SOLUTION

Antibiotic stock solution can be prepared using the formula

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

Where P= potency of the antibiotic in relation to the base. (For Vancomycin

P= 950/1000 µg)

V = volume of the stock solution to be prepared (10ml)

C = final concentration of the antibiotic solution (1024µg/ml)

W = weight of the antibiotic to be dissolved in the volume V

PEAPARATION OF DRUG DILUTIONS

Two rows of sterile test tubes were arranged one for the test and the other for the ATCC control with 1 ml of MH broth. From the stock solution (tube 1) 1ml was transferred to the second tube, then from the second to the third tube. This procedure was repeated till the thirteenth tube. Growth control and the sterility control for the antibiotic solution was also kept.

Inoculum preparation for the test and ATCC control and incubation

To 9.9 ml of MH broth 0.1ml of 0.5 Mcfarland turbidity matched test isolate suspension was added, mixed well and from this 1 ml of inoculum was transferred to each tube containing antibiotic dilutions and also to the control tube. Same procedure was repeated for ATCC control strain and incubated at 37ºC – overnight. MIC of ATCC control strain and the test organism was observed. The lowest concentration of the antibiotic in which there is no visible growth is considered as the MIC for the ATCC strain & for the test organisms.

TABLE -10 SERIAL DRUG DIUTION AND DRUG CONCENTRATION

Tubes	**1 [Stock]**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Amount(ml) of MH broth to be added	2	1	1	1	1	1	1	1	1	1	1	1	1
Serial dilution of drug-1 ml to be transferred		From 1	From 2	From 3	From4	From 5	From6	From 7	From8	From9	From 10	From 11	From 12
Drug concentration(µg/ml)	1024	512	256	128	64	32	16	8	4	2	1	0.5	0.25
Addition of inoculum (1 ml)		1	1	1	1	1	1	1	1	1	1	1	1
concentration		256	128	64	32	16	8	4	2	1	0.5	0.25	0.125

Interpretation:

Only if the MIC of the ATCC strain is in the sensitivity range the MIC for the test isolate is read otherwise test is invalid. The MIC for the drug and the test isolate is the lowest concentration of the antibiotic in which there is no visible growth.

TABLE-11-A MIC range for Vancomycin by macrobroth dilution method

VANCOMYCIN	RESISTANT µg/ml	INTERMEDIATE µg/ml	SENSITIVE µg/ml
S.aureus	≥32	8-16	≤4
Enterococci.spp	≥16	4-8	≤2

ANTIFUNGAL SUSCEPTIBILITY

DISC DIFFUSION METHOD

Isolates to be tested were subcultured on SDA to obtain pure and viable cultures. Inoculum was prepared by suspending 4-5 colonies of 24 hour old culture in 5 ml sterile NaCl (0.85%) and adjusted to 0.5 McFarland's turbidity standard.

Within fifteen minutes of preparation of the suspension, a sterile cotton-wool swab was dipped into the suspension and the surplus was removed by rotating the swab against the side of the test tube.

With this swab, the Mueller Hinton agar supplemented with 2% glucose and 0.5 µg/ml methylene blue plate was inoculated by even stroking of the swab over the entire surface of the plate in three directions so as to obtain a lawn culture.

After brief drying, the antibiotic disc was placed on each plate. With each batch of tests, a control for each antibiotic was also set up.

The control strains were obtained from American Type Culture Collection (ATCC). The control strains were included as per the CLSI guidelines. Antifungal discs used were Fluconazole [25µg] and voriconazole [1µg].

TABLE-12 Antifungal susceptibility and their interpretative criteria

ANTIFUNGAL	DISC POTENCY	ZONE DIAMETR RANGE		
		R	SDD	S
FLUCONAZOLE	25µg	≤14	15-18	≥19
VORICONAZOLE	1µg	≤13	14-16	≥17

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION [MIC] BY MICRO BROTH DILUTION METHOD

As per M27-A3 document in CLSI guidelines MIC for yeast is detected.

MEDIA- RPMI [Rose Well Park Memorial Institute] 1640 medium with L-glutamine, without sodium bicarbonate buffered with MOPS[3-(N-morpholino) propane sulfonic acid].To dissolve water insoluble drugs DMSO[dimethyl sulfoxide] is used.

INOCULUM PREPARATION

Isolates to be tested were subcultured on SDA to obtain pure and viable cultures. Inoculum was prepared by suspending 4-5 colonies of 24 hour old culture in 5 ml sterile NaCl [0.85 %], vortexed , adjusted to 0.5 McFarland's turbidity standard at 530 nm wavelength in a spectrophotometer and further diluted 1:50 with the standard medium so that the test inoculum is $1x\ 10^3$ to $5\ x\ 10^3$ CFU/ml.

Weight of antifungal drugs

$$\text{Weight (mg)} = \frac{\text{volume(ml) x concentration (µg/ml)}}{\text{Assay potency (µg/mg)}}$$

PREPARATION OF STOCK SOLUTION

Water soluble drug fluconazole dissolved in distilled water in the concentration of 5120 µg/ml. Water insoluble drugs like itraconazole, ketoconazole and voriconazole dissolved in DMSO in the concentration of 1600 µg/ml.

INTERMEDIATE DRUG CONCENTRATION

WATER INSOLUBLE DRUGS

Ten tubes arranged in a row. Stock solution 1600 µg was taken in first tube. 0.5 ml of DMSO in the second, fifth and eighth tubes, 1.5 ml of DMSO in the third, sixth and nineth tubes and 3.5 ml of DMSO in fourth, seven and tenth tubes were added.

From the first tube 0.5 ml of the drug was transferred to second, third and fourth tubes. From the fourth tube 0.5ml was transferred to fifth, sixth and seventh tubes. From the seventh tube 0.5 ml was transferred to eight, nineth and tenth tubes.

WATER SOLUBLE DRUGS

Ten tubes were arranged in a row. 7 ml of RPMI was added to the first tube. 1ml of RPMI was added to second, fourth, seventh and tenth tubes. 3 ml of RPMI was added to third tube. 1.5 ml of RPMI was added to fifth and eighth tubes. 3.5 ml of RPMI was added to the sixth and nineth tubes.

1ml of drug was added to the first tube [stock].From the first tube 1 ml was transferred to second, third and fourth tubes. From the fourth tube 0.5 ml was transferred to fifth and sixth tubes. From the sixth tube 1 ml was transferred to seventh tube and 0.5 ml to the eighth and nineth tube. From the nineth tube 1ml was transferred to tenth tube.

FINAL DRUG CONCENTRATION

WATER INSOLUBLE DRUG

Another row of ten tubes are arranged and 4.9 ml of RPMI added in all the tubes. To these 0.1ml of intermediate drug prepared were added from the corresponding rows.

WATER SOLUBLE DRUG

Another row of ten tubes are arranged and 4 ml of RPMI added in all the tubes. To these 1ml of intermediate drug prepared were added from the corresponding rows.

METHOD100µl of each serial dilution of antifungal agents and a constant volume100µl of inoculum were added in the wells of flat bottomed 96 well microtitre plate. Incubated at 35º C for 48 hours. Growth and drug control were included.

TABLE-13 Antifungal drug concentrations for water insoluble drugs

TUBES (water insoluble)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Intermediate drug concentration	1600	800	400	200	100	50	25	12.5	6.25	3.13
Final drug concentration	32	16	8	4	2	1	0.5	0.25	0.125	0.0625
drug concentration after adding inoculum	16	8	4	2	1	0.5	0.25	0.125	0.0625	0.0313

TABLE-14 Antifungal drug concentrations for water insoluble drugs

TUBES **(water soluble)**	1	2	3	4	5	6	7	8	9	10
Intermediate drug concentration	640	320	160	80	40	20	10	5	2.5	1.25
Final drug concentration	128	64	32	16	8	4	2	1	0.5	0.25
drug concentration after adding inoculum	64	32	16	8	4	2	1	0.5	0.25	0.125

Growth control was added to column 11, with 100µl of RPMI and 100µl of inoculum. Drug control was added to column 12 with 100µl of RPMI and 100µl of drug.

INCUBATION

The microtitre plate was incubated at 35°C for 24 hours for Candida species.

Reading of results and scores:

1- Optically clear absence of growth
2- Approximately 25% of the growth control or Slight growth.
3- Approximately 50% of the growth control or prominent reduction in growth.
4- Approximately as of the growth medium or slight reduction in the growth.
5- No reduction in the growth.

INTERPRETATION

The MIC for azole drugs was the lowest concentration with a score of 3. This corresponds to approximately 50% of the growth control or prominent reduction in growth.

TABLE-11-B MIC range for Azole drugs by macrobroth dilution method

Candida .spp	**RESISTANT µg/ml**	**SUSCEPTIBLE DOSE RANGE µg/ml**	**SENSITIVE µg/ml**
Fluconazole	≥64	16-32	≤8
Itraconazole	≥1	0.25-0.5	0.125
Voriconazole	≥4	2	≤1

MOLECULAR METHOD:

Polymerase chain reaction:

The isolates of *E.coli* which were having > 10^5 CFU/ml by semiquantitative culture method were subjected to conventional PCR for the detection of ompT gene.[]

DNA extraction:

5-10 colonies of*E.coli*were inoculated into nutrient broth andincubated overnight at 37ºC. 1.5ml of overnight broth culture was transferredinto 1.5ml of centrifuge tube and centrifuged at 10,000 rpm for 3 minutes. Supernatant was discarded, excess medium was removed by gently tappingthe tube on a paper towel.

Procedure:

1. The pellet obtained was suspended in 200µl of PBS.
2. 180µl of Lysozyme digestion buffer and 20µl of Lysozyme were added.
3. Above mixture was mixed well and incubated at 37 ºC for 15min.
4. After incubation 200µl of Lysis buffer and 20µl of Proteinase K [10mg/ml] were added and incubated at 56ºC for 10min in waterbath.
5. Then 300µl of Isopropanol was added and mixed well.
6. The whole lysate was transferred into PureFast spin column and centrifuged at 10000rpm for 1min.
7. Flow through was discarded and 500µl of Wash buffer-1 was added to spin column and centrifuged at 10000rpm for 1min.
8. Flow through was discarded and 500µl of Wash buffer-2 was added to spin column and centrifuged at 10000rpm for 1min. washing was repeated one more time.
9. Flow through was discarded and the column was centrifuged for additional 2 minutes to remove any residual ethanol.
10. The DNA was eluted by adding 100µl of Elution buffer and centrifuged for 1min. The eluted DNA was used as the template for PCR.

Primers: [Designed by HELINI Biomolecules, Chennai]

GENE	PRIMER SEQUENCES	AMPLICON SIZE IN BASE PAIRS
ompT	5'-CACGCTCCACAAACCAAGTG-3	**1120**

PCRProcedure:

1. Reactions were set up as follows;

Components	Quantity
HELINI 2X PCR Master Mix	10µl
Primer Mix 10pmoles/Reactions	5µl
Genomic DNA	5µl
Total volume	20µl

2. All the components were mixed gently and placed into PCR machine (Corbett thermocycler) and programmed it as follows,

Cycle Number	Denaturation	Annealing	Extension
1	94ºC for 5 min	-	-
35	94ºC for 30sec	58ºC for 30sec	72ºC for 30sec
1	-	-	72º C for 5 min

Agarose gel electrophoresis:

1. 2% agarose gel was prepared [2gm of agarose in 100ml of 1x TAE buffer] with eight wells.
2. 8µl 6X Gel loading dye was mixed to each PCR vial.
3. 15µl from each PCR vial was loaded.
4. Then 100bp and 1000 bp DNA ladder and appropriate controls were loaded.
5. Electrophoresis was run at 50V till for 40 min and the bands were observed using UV Transilluminator.

Interpretation:

The amplified PCR products and 100bp 1000 bp DNA molecular markerswere seen as bright fluorescent bands with satisfactory controls. A 1120bp corresponds to ompT gene.

Results

RESULTS

A total of 600 midstream urine samples were processed from 100 patients each undergoing cystoscopy and urodynamic procedure attending urology OP, Rajiv Gandhi Government General Hospital, Chennai.

From each patient 3 urine samples were collected on the day 0 (just before the procedure), day 3 (3 days after the procedure) and day 7 (7 days after the procedure). So 300 samples were collected from 100 patients each undergoing cystoscopy and urodynamic procedure accounting to 600 samples.

Samples collected just before the procedure (day 0) which were culture positive were subsequently excluded from the study. Patient selection criteria will be based on only when the midstream urine samples is declared negative for urinary tract infection.

The results will be discussed separately for the two groupsi.e cystoscopy and urodynamic procedure.

CYSTOSCOPY

TABLE-15 Age and Sex wise distribution of samples collected from cystoscopy patients

Age	Male	Female	Total
21-30	4 (7.27%)	1 (2.22%)	5(5%)
31-40	8 (14.5%)	3 (6.66%)	11 (11%)
41-50	13 (23.6%)	11 (24.4%)	24 (24%)
51-60	16 (29%)	20 (44.4%)	36 (36%)
61-70	7 (12.7%)	8 (17.7%)	15 (15%)
71-80	7 (12.7%)	2 (4.44%)	9 (9%)
Total	55 (55%)	45 (45%)	100

Among the100 patients underwent cystoscopy 55(55%) of them were male patients and 45 (45%)of them were female patients. Majority of the patients were from the 51-60 (36%) age group followed by the 41-50 (24%) age group in the both genders. Least number of patients were from <30 (5%) age group and the 71-80 (9%) age group in the both genders.

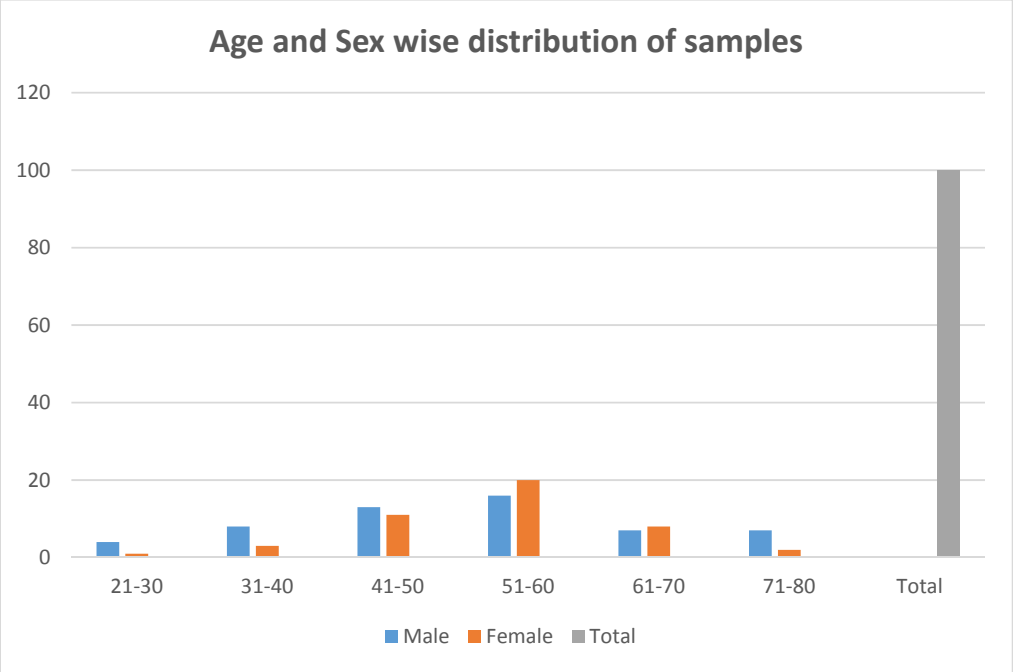


TABLE-16 Age and Sex wise distribution of culture positive patients

Age	Male		Female		Total		Total Culture +ve patients (%)
	No. of patients	No. of Culture +ves	No. of patients	No. of Culture +ves	No. of patients	Culture +ve patients	
21-30	4	1 (25%)	1	1(100%)	5	2	40
31-40	8	2 (25%)	3	1(33.33%)	11	3	27.27
41-50	13	6 (46%)	11	5(45.55%)	24	11	45.83
51-60	16	6 (37.5%)	20	7(25.9%)	36	13	36.11
61-70	7	3 (42.8%)	8	2(25%)	15	5	33.33
71-90	7	3 (42.8%)	2	1(50%)	9	4	44.44
Total	55	21(38.18%)	45	17(37.77%)	100	38	38

Above table shows that 38 patients (38%) were culture positive among 100 patients underwent cystoscopy. Among these 38 culture positives 21 (38.18%) were males and 17 (37.77%) were females. Majority of the culture positive patients were between 41-50 (45.83%) age group in the both genders.

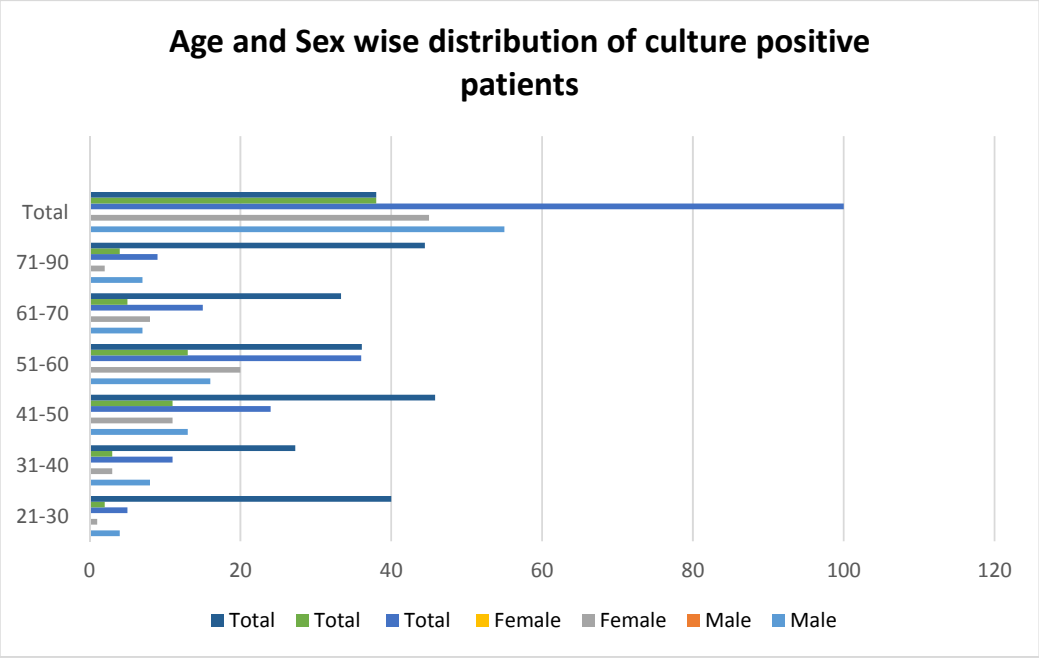


Table- 17 Correlation between pyuria, Gram's stain and culture

Gram's stain showing bacteria	Growth on culture	Wet mount No. of pus cells/HPF [High power field]			Total samples -300	Total %
		0-3/HPF	3-5/HPF	>5/HPF		
Absent	Absent	243	15	2	260	86.66
	Present	-	2	29	31	10.33
Present	Present	-	2	7	9	3

Above table shows that among 300 samples only 9(3%) samples showed bacteria on Gram stain and the remaining 291 samples does not show any bacteria on Gram stain. Among the 291 samples 260(86.66%) samples were culture negative and 31 (10.33%) samples were culture positive. Among the 260 samples 243 samples had 0-3 pus cells, 15 samples had 3-5 pus cells and 2 samples had >5 pus cells per high power field. Among the 31 samples 2 sample had 3-5 pus cells and 29 samples had > 5pus cells per high power field. All the 9 samples showing bacteria on Gram stain were culture positive. Among these 2 samples had 3-5 pus cells and 7 samples had > 5 pus cell per high power field.

Chart-3 **Correlation between pyuria, Gram's stain and culture**

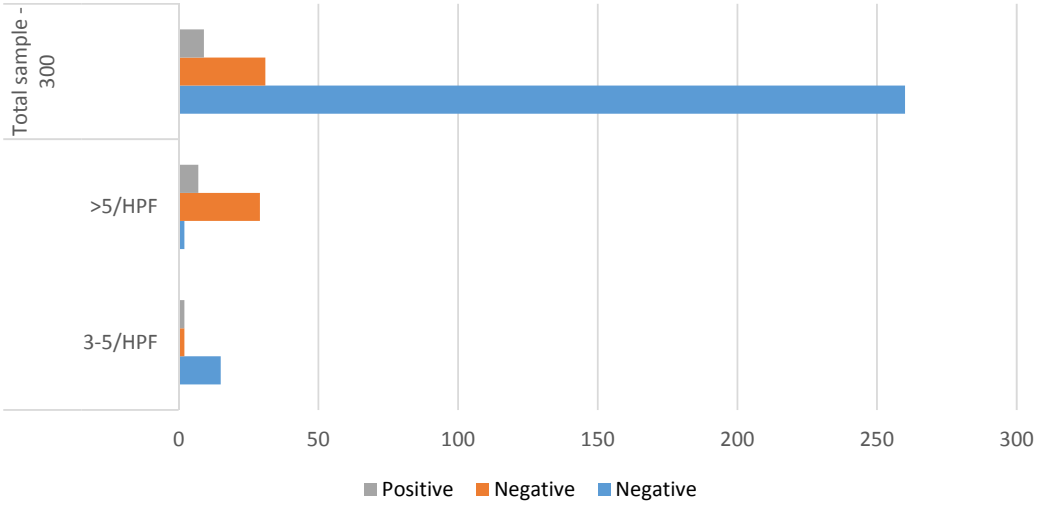


Table-18 Distribution of Culture +ve and Culture –ve patients

Gender	No.of pts	Culture +ve Pts			Culture -ve Pts
		No.of Pts with Single isolate	No.of Pts with Mixed isolates(2 isolates)	Total No.ofCulture+ve Pts	
Male	55	19	2	21	34
Female	45	14	3	17	28
Total	100	33(33%)	5(5%)	38(38%)	62(62%)

Among the total 100 patients 38(38%) patients were culture positive and 62 (62%) patients were culture negative. Among the 38 culture patients 33 (33%) were culturepositive for single organism on culture and 5 (5%) were culture positive for mixed organisms.

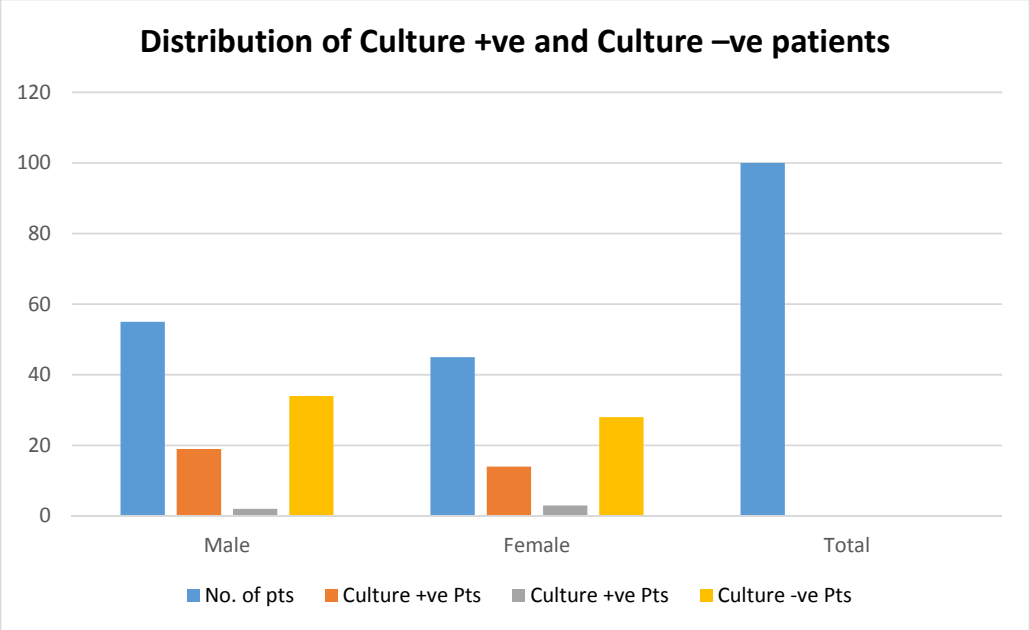


Table-19 Daywise analysis for Culture +ve single organism

	No. of pts	Culture +ve for single organism		
		3rd day+ve 7th day-ve	**3rd day-ve 7th day+ve**	**3rd day+ve 7th day+ve**
Male	19	15	3	1
Female	14	9	4	1
Total	33	24(24%)	7(7%)	2(2%)

Among the 33 culture positive patients for single isolates 19 patients were from males and 14 patients were from females. Majority of the patients yielded growth on the third day. 24 patients yielded growth on third day and these patient were culture negative on seventh day, 7 patients who yielded no growth on third day were yielded growth on seventh day and only 2(2%) patients yielded growth on both third and seventh day.

Table-20 Daywise analysis for Culture +ve for mixed organism

	No. of pts	Culture +ve for mixed organism(2 isolates)		
		3rd day+ve 7th day-ve	**3rd day-ve 7th day+ve**	**3rd day+ve 7th day+ve**
Male	2	2	-	0
Female	3	2	1	0
Total	5(5%)	4(4%)	1(1%)	0

Among the 5(5%) culture positive patients for mixed isolates 2 patients were from males and 3 patients were from females. Majority of the patients yielded growth on the third day. 4(4%) patients yielded growth on third day, 1 (1%)patient yielded growth on seventh day. None of patients yielded mixed growth on both third and seventh day.

Table- 21 Day wise distribution of single isolates

Isolates	**Total**	**3rd day+ve 7th day-ve**	**3rd day-ve 7th day+ve**	**3rd day+ve 7th day+ve**
E.coli	12	8	2	2
Klebsiella.spp	5	4	1	-
Proteus .spp	6	5	1	-
Acinetobacter.spp	3	2	1	-
Enterococci	2	1	1	-
Pseudomanasaeruginosa	2	2	-	-
Candida. Spp	2	1	1	-
Staph.aureus	1	1	-	-
Total	33	24	7	2

Majority of them were isolated on third day i.e 24 out of 33 (72.7%), 7 were isolated on seventh day (21.2%) and 2 were (6%) isolated on both third and seventh day.

Table-22 Day wise distribution of mixed isolates

No of Culture +ve patients for mixed organism(2 isolates)			
Mixed Isolates	**3rd day+ve 7th day-ve**	**3rd day-ve 7th day+ve**	**3rd day+ve 7th day+ve**
E.coli+Enterococci E.coli+Klebsiellaspp	1 1	-	0
E.coli+ Enterococci E.coli+CONS	1 1	-	0
E.coli+C.albicans	-	1	0
Total	4(80%)	1(20%)	0

Out of five patients four (80%) patients yielded two isolates on third day, 1 (20%) patient yielded two isolates on seventh day and none of the patients yielded mixed isolates on both third and seventh day.

Table- 23 Day wise distribution of single and mixed isolates

Isolates	**Total**	**3rd day+ve 7th day-ve**	**3rd day-ve 7th day+ve**	**3rd day+ve 7th day+ve**
E.coli	17	13	2	2
Klebsiella.spp	6	5	1	-
Proteus .spp	6	5	1	-
Acinetobacter.spp	3	2	1	-
Enterococci	4	2	2	-
CONS	1	1	-	-
Pseudomanasaeruginosa	2	2	-	-
Candida. Spp	3	2	1	-
Staph.aureus	1	1	-	-
Total	43	33	8	2

The table above shows that among the total 43 isolates 33(76.67%) were isolated on the third day, 8(18.6%)were isolated on the seventh day and 2 (4.6%) were isolated on both third and seventh day.

Majority of the isolates were *E.coli* (39.5%) followed by *Klebsiella .spp* (13.33 %), *Proteus .spp* (13.33 %), *Enterococci* (8.89%), *Acinetobacter.spp*(6.67%), *Candida. Spp*(6.67%), *Pseudomonas aeruginosa* (4.44%), *Staphylococcus aureus* (2.22%) and *CONS* (2.22 %).

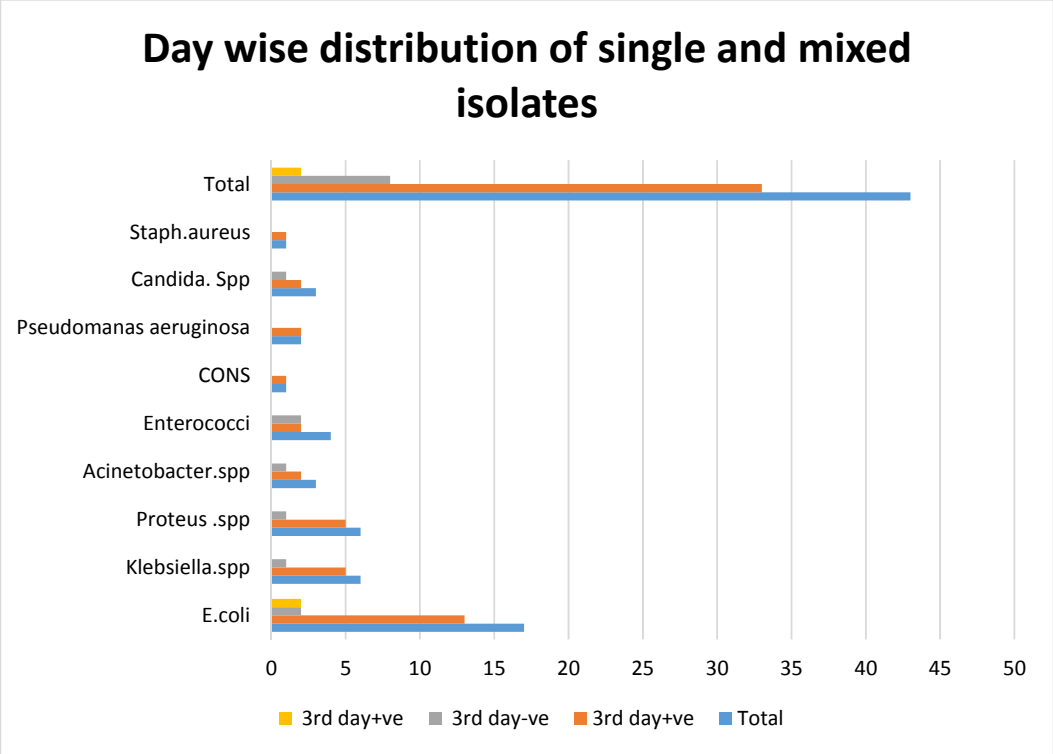


Table -24 Distribution of isolates among different age groups

Isolates	Age group						Total isolates
	21-30	31-40	41-50	51-60	61-70	71-90	
E.coli	2	-	4	8	2	1	17
Klebsiella.spp	-	2	1	-	3	-	6
Proteus .spp	-	-	2	3	1	-	6
Enterococci	-	-	1	-	1	2	4
Acinetobacter.spp	-	-	1	-	-	2	3
Candida.spp	-	-	2	1	-	-	3
Pseudomonas aeruginosa	1	-	-	-	-	1	2
CONS	-	-	1	-	-	-	1
Staph.aureus	-	1	-	-	-	-	1
Total	3 (6.9%)	3 (6.9%)	12 (27.9 %)	12 (27.9 %)	7 (16.2%)	6 (13.9%)	43

The table above shows that out of 43 isolates majority of the isolates were from the 41-50 (27.9 %) and 51-60 age group (27.9 %) followed by 61-70 (16.2%)and 71-90 (13.9%)age group. Least isolates were from the 20-30 and 31-40 (6.9%) age group.

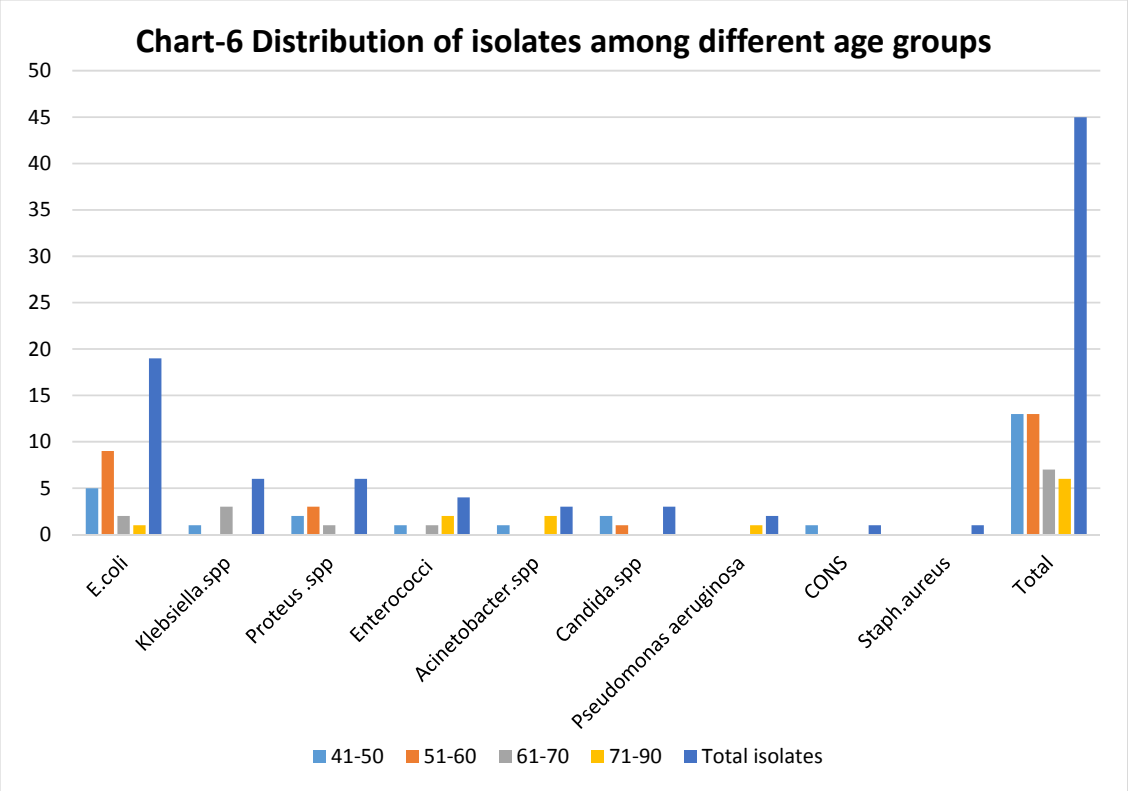


Table-25 Colony counts of isolates

Isolates	Colony count- CFU/ml				Total isolates
	$<10^2$	10^2-10^4	10^4- 10^5	$>10^5$	
E.coli	5	6	3	3	17
Klebsiella.spp	2	4	-	-	6
Proteus .spp	-	3	2	1	6
Enterococci	-	2	1	1	4
Acinetobacter.spp	-	1	2	-	3
Candida.spp	1	2	-	-	3
Pseudomonas aeruginosa	-	1	1	-	2
CONS	-	1	-	-	1
Staph.aureus	-	1	-	-	1
Total	9	20	9	5	43

The table above shows that 5 patients (5%) out of 100 patients had significant colony count (>10^5 CFU/ml). Majority of the patients had colony counts inbetween 10^2-10^4 CFU/ml (20 %).

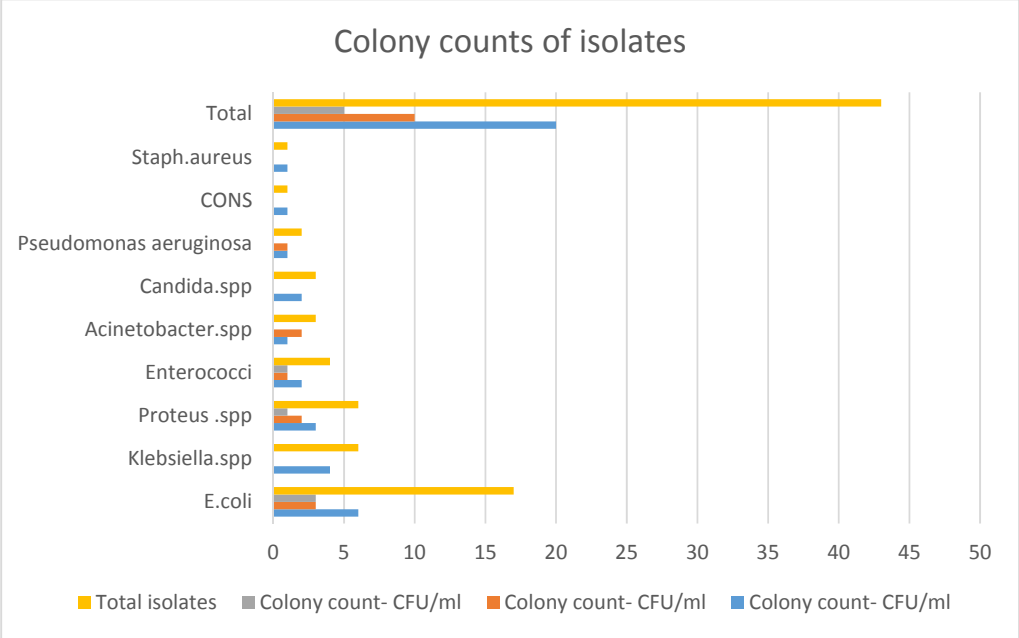


Table-26 Daywise analysis of significant colony count (>10^5cfu/ml)

Isolates	3rd day+ve 7th day-ve	3rd day-ve 7th day+ve	3rd day+ve 7th day+ve	Total
E.coli	1	-	2	3
Proteus .spp	1	-		1
Enterococci	1	-		1
Total	3(3%)	0	2(2%)	5(%)

Among the 100 patients only 5 (5%) patients had more than 10^5cfu/ml .Three patientson the third day and two (2%)patients on both third day and seventh day had significant colony count.

Table-27 Antibiogram of isolates – Cystoscopy -Bacterial isolates

Isolates	AK		GM		NF		NX		CTX		AMP	
	S	R	S	R	S	R	S	R	S	R	S	R
17-*E.coli*	16 (94.1%)	1 (5.8%)	7 (41.1%)	10 (58.2%)	15 (88.2%)	2 (11.7%)	16 (94.1%)	1 (5.8%)	15 (88.2%)	2 (11.7%)	7 (41.1%)	10 (58.2%)
6-*Klebsiella.spp*	5 (83.33%)	1 (16.66%)	5 (83.33%)	1 (16.66%)	5 (83.33%)	1 (16.66%)	5 (83.33%)	1 (16.66%)	5 (83.33%)	1 (16.66%)	1 (16.66%)	5 (83.33%)
6-*Proteus spp*	6 (100 %)	0 0	4 (66.67%)	2 (33.33%)	4 (66.67%)	2 (33.33%)	5 (83.33%)	1 (16.66%)	5 (83.33%)	1 (16.66%)	2 (33.33%)	4 (66.67%)

Table-27 –A Gram negative bacilli –ENTEROBACTERIACEAE NON-ENTEROBACTERIACEAE

Isolates	AK		GM		NX		CIP		CZ		PT	
	S	R	S	R	S	R	S	R	S	R	S	R
2-*Pseudomonas aeruginosa*	2(100%)	0	0(0%)	2(100%)	2(100%)	0	0(0%)	2(100%)	2(100%)	0	2(100%)	0
3-*Acinetobacter.spp*	3(100 %)	0	2(66.66%)	1(33.33%)	3 (100 %)	0	2(100 %)	1(33.33%)	3(100 %)	0	3(100 %)	0

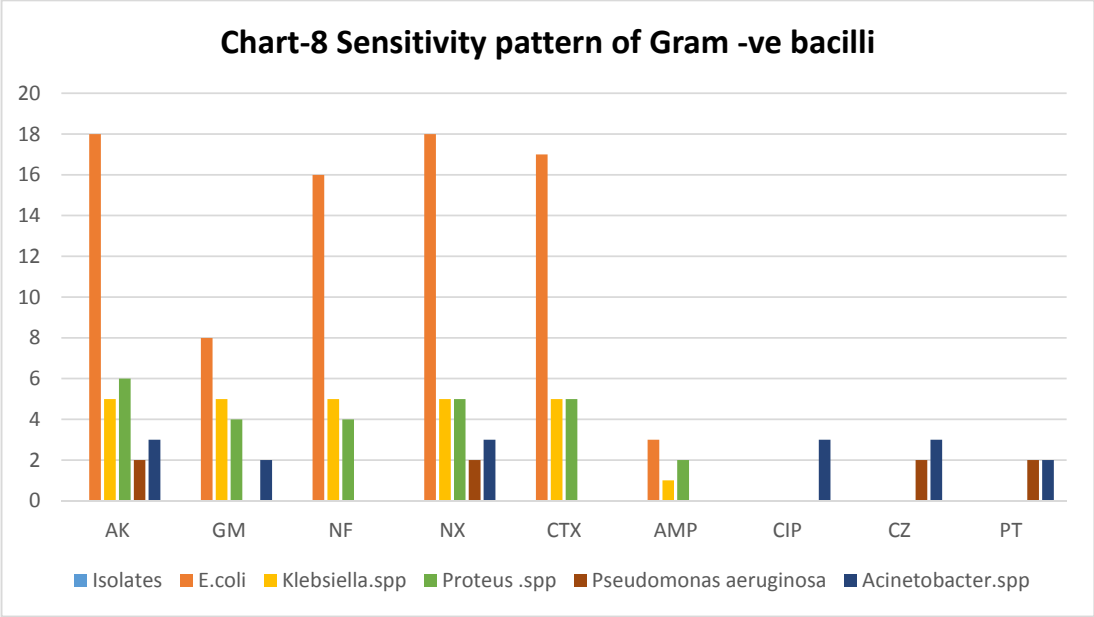


Table-27 –B Gram positive cocci

S.aureus

Isolates	NF		COTRI		PEN		ERY		CX		NX	
	S	R	S	R	S	R	S	R	S	R	S	R
1-*Staph.aureus*	1 (100%)	0	1 (100%)	0	0 (0%)	(100%)	1 (100%)	0	1 (100%)	0	1 (100%)	0

Enterococci

Isolates	NF		PEN		NX		HLG		CIP		AMPI	
	S	R	S	R	S	R	S	R	S	R	S	R
4-*Entero-cocci*	2 (50%)	2 (50%)	0	4 (100%)	2 (50%)	2 (50%)	4 (100%)	0	1 (25%)	3 (75%)	2	2 (50%)

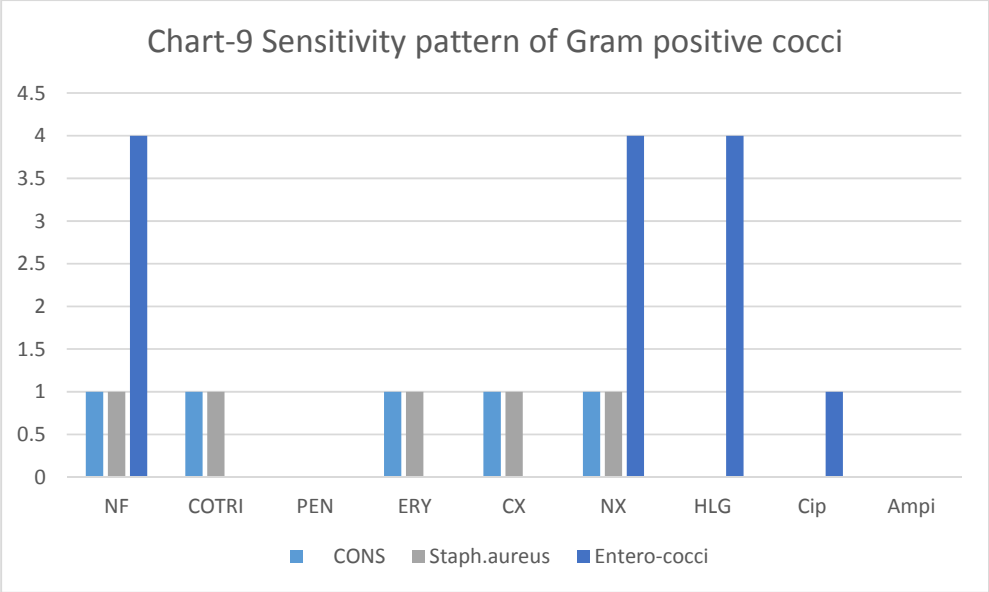
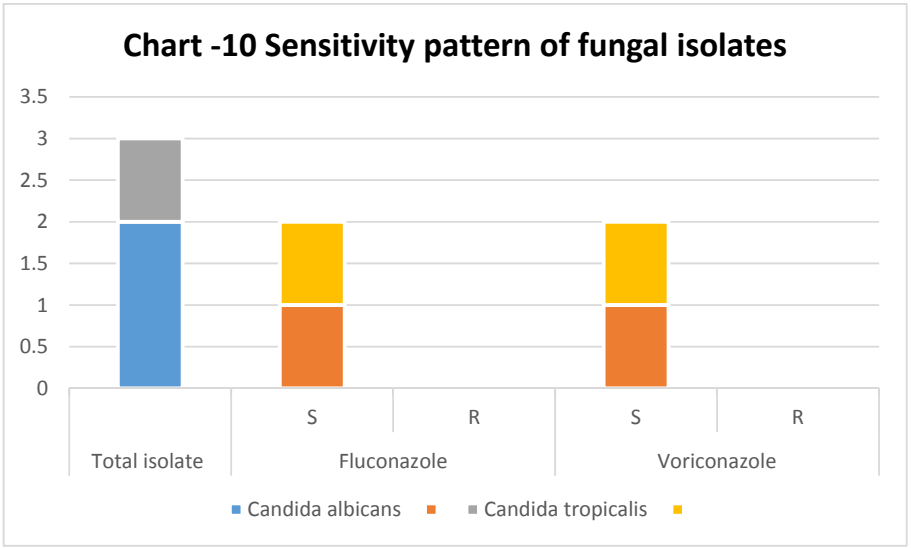


Table-2 –C Sensitivity pattern of fungal isolates

Isolate	Total isolate	Fluconazole		Voriconazole	
		S	R	S	R
Candida albicans	2	2	0	2	0
Candida tropicalis	1	1	0	1	0



URODYNAMIC STUDY

TABLE-28 Age and Sex wise distribution of samples

Age	Male	Female	Total(male+female)
21-30	2(3.7%)	2(4.3%)	4(4%)
31-40	7(12.9 %)	7(15.2%)	14(14%)
41-50	17(31.4 %)	12(26%)	29(29%)
51-60	9(16.6%)	14(30.4%)	23(23%)
61-70	12(22.2%)	8(17.3%)	20(20%)
71-90	7(12.9%)	3(6.5%)	10(10%)
Total	54(54%)	46(46%)	100

Above table shows that among the 100 patients underwent urodynamic study 54 of them were male patients (54%) and 46 of them were female patients (46%). Majority of the patients were from 41-50 (29%) age group followed by 51-60 age group (23%), 61-70 (20%) age group followed by 31-40 (14%)age group. Least number of patients from 21-30(4%) age group.

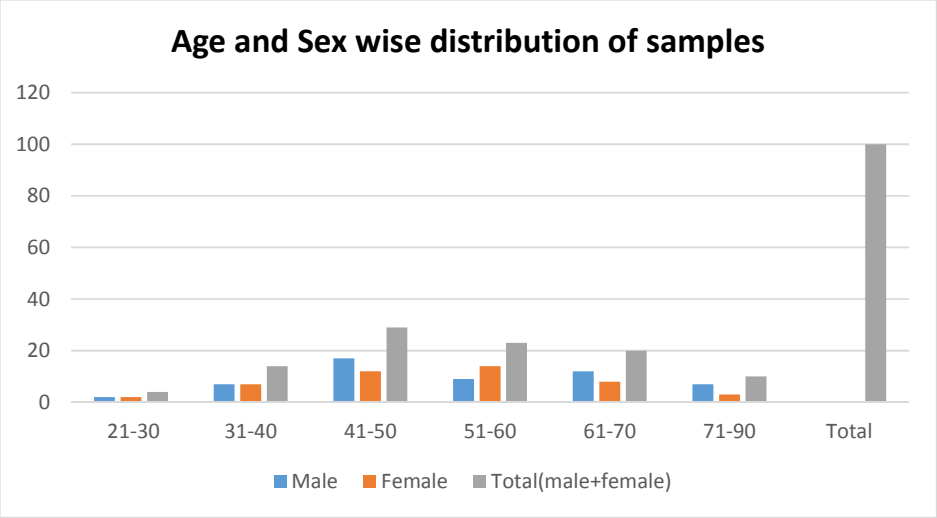


TABLE-29 Age and Sex wise distribution of culture positive patients –

Age	Male		Female		Total Culture +ve patients		Total Culture +ve%
	Total No. of patients	Culture +ves patients	Total No. of patients	Culture +ves patients	No. of patients	Culture +ve patients	
21-30	2	0	2	1 (50%)	4	1	25
31-40	7	2 (28.5%)	7	2 (28.5%)	14	4	28.57
41-50	17	1 (5.88%)	12	1 (5.8%)	29	2	6.90
51-60	9	3 (33.3%)	14	2 (14.2%)	23	6	21.74
61-70	12	1 (8.3%)	8	1 (12.5%)	20	2	10
71-90	7	1 (14.2%)	3	0	10	1	10
Total	54	8 (14.8%)	46	7 (15.2%)	100	15	15

Above table shows that 15 (15%) patients were culture positive among 100 patients underwent urodynamic study. Among these 15 culture positives 8 (14.8%) were males and 7 (15.2%) were females.

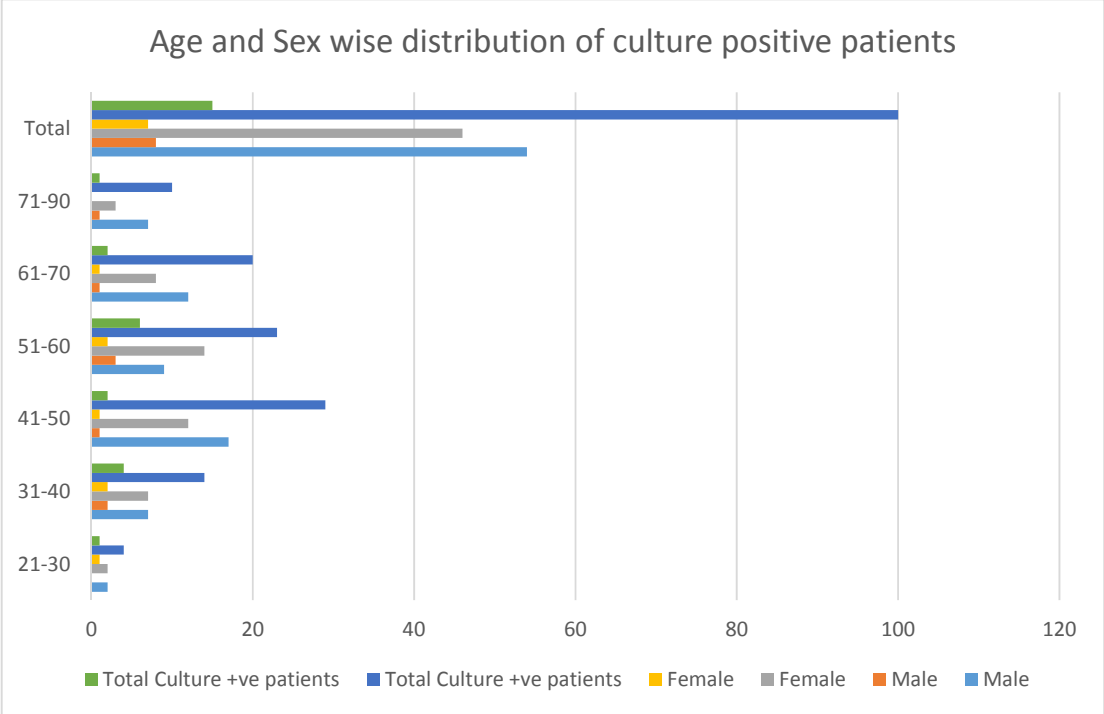


Table-25 Correlation between pyuria, Gram's stain and culture

Gram's stain showing bacteria	Growth on culture	Wet mount No. of pus cells/HPF			Total samples-300
		0-3/HPF	3-5/HPF	>5/HPF	
Absent	Absent	273	11	-	284(94.6%)
	Present	-	9	2	11(3.6%)
Present	Present	-	1	4	5(1.6%)

Among 300 samples only 5 (1.6%) samples showed bacteria on Gram stain and the remaining 295 samples does not show any bacteria on Gram stain. Among the 295 Gram stain ne samples 284 samples (94.6%) were culture negative and 11 sample was culture positive. Among the 284 samples 273 samples had 0-3 pus cells and 11samples (3.6%)had 3-5 pus cells per high power field. Among the 11samples 2 had > 5 pus cells and 9 had 3-5 pus cells per high power field. All the 5 samples showing bacteria on Gram stain were culture positive. Among these 5 samples 1 had 3-5 pus cells and 4 samples had > 5 pus cell per high power field.

Chart-12 Correlation between pyuria, Gram's stain and culture

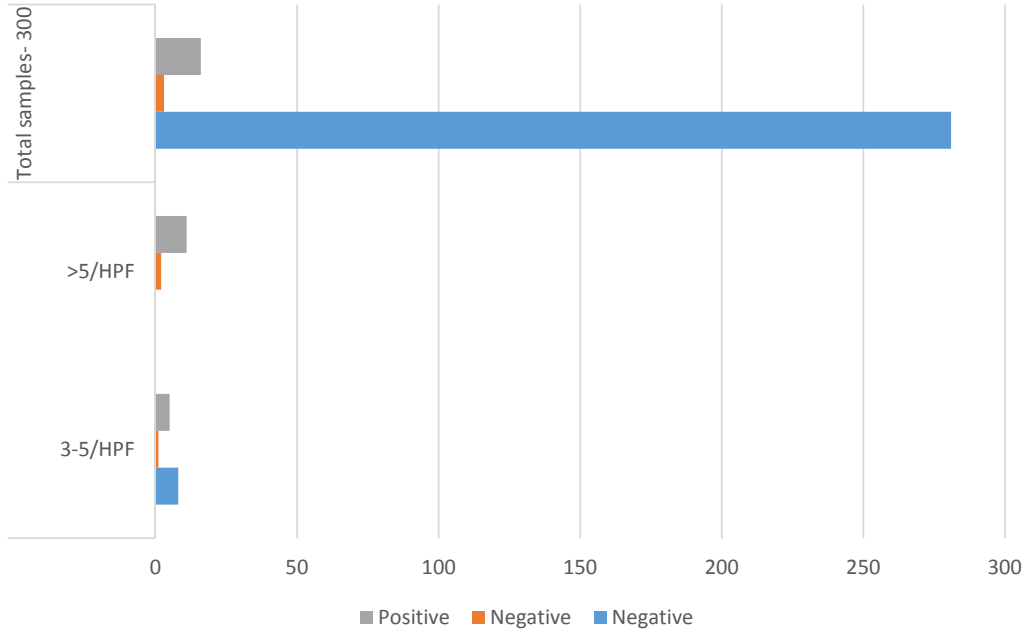


Table-18 Distribution of Culture +ve and Culture –ve patients

Gender	No. of pts	Culture +ve Pts			Culture -ve Pts
		No.of Pts with Single isolate	No.of Pts with Mixed isolates(2 isolates)	Total No.ofCulture +ve Pts	
Male	54	7	1	8	46
Female	46	5	2	7	39
Total	100	12(12%)	3(3%)	15(15%)	85(85%)

Among the total 100 patients 15(15%) patients were culture positive and patients 85(85%) were culture negative. Among the 15 culture patients 12(12%) were culture positive for single organism on culture and 3 (3%) were culture positive for mixed organisms.

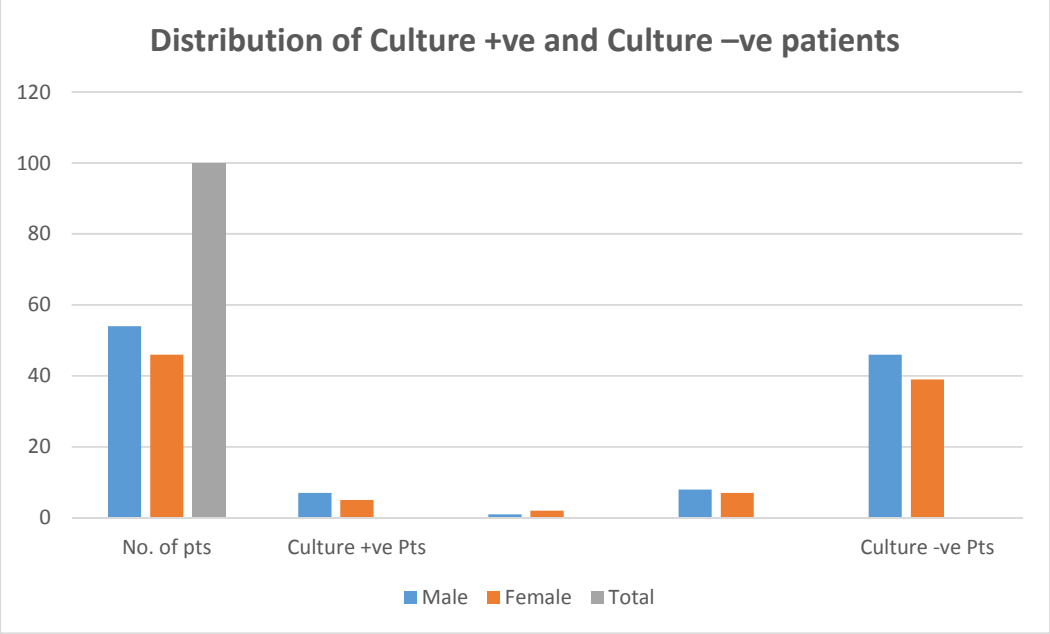


Table-19 Daywise analysis for Culture +ve for single organism

	No. of Culture +ve pts	Culture +ve for single organism		
		3^{rd}day+ve 7^{th} day-ve	3^{rd} day-ve 7^{th}day+ve	3^{rd}day+ve 7^{th}day+ve
Male	7	5	1	1
Female	5	3	1	1
Total	12	8(66.66%)	2(16.66%)	2(16.66%)

Among the 12 culture positive patients for single isolates 7 patients were from males and 5 patients were from females. Majority of the patients yielded growth on the third day. 8 (66.66%) patients yielded growth on third day, 2(16.66%) patients yielded growth on seventh day and 2 (16.66%)patients yielded growth on both third and seventh day.

Table-20 Daywise analysis for Culture +ve for mixed organism

	No. of Culture +ve pts	No. of Culture +ve Pts for mixed organisms (2 isolates)		
		3^{rd}day+ve 7^{th} day-ve	3^{rd} day-ve 7^{th}day+ve	3^{rd}day+ve 7^{th}day+ve
Male	1	1	0	0
Female	2	1	1	0
Total	3	2(66.66%)	1(33.33%)	0

Among the 3 culture positive patients for mixed isolates 1 patient were from males and 2 patients were from females. 2 (66.66%) patients yielded growth on third day, 1(33.33%) patient yielded growth on seventh day. None of patients yielded mixed growth on both third and seventh day.

Table- 21 Day wise distribution of single isolates

Isolates	Total	3rd day+ve 7th day-ve	3rd day-ve 7th day+ve	3rd day+ve 7th day+ve
E.coli	6	4	1	1
Klebsiella.spp	3	2	-	1
Proteus .spp	2	1	1	-
Enterococci	1	1	-	-
Total	12	8(66.66%)	2(16.66%)	2(16.66%)

Majority of them were isolated on third day i.e 8 out of 12(66.66%), 2 were isolated on seventh day (16.66%) and 2 were (16.66%) isolated on both third and seventh day.

Table-22 Day wise distribution of mixed isolates

Mixed isolates	Culture +ve for mixed organism(2 isolates)		
	3rd day+ve 7th day-ve	**3rd day-ve 7th day+ve**	**3rd day+ve 7th day+ve**
E.coli+Proteusspp	1	-	0
Klebsiellaspp +CONS	1	-	0
E.coli+C.albicans	-	1	0

Out of three patients two patients yielded two isolates on third day, 1 patient yielded two isolates on seventh day and none of the patients yielded mixed isolates on both third and seventh day.

Table- 23 Day wise distribution of single and mixed isolates

Isolates	3rd day+ve 7th day-ve	3rd day-ve 7th day+ve	3rd day+ve 7th day+ve	Total
E.coli	5	2	1	8(44.4%)
Klebsiella.spp	3		1	4(22.2%)
Proteus .spp	2	1	-	3(16.6%)
Enterococci	1	-	-	1(5.5%)
CONS	1	-	-	1(5.5%)
Candida. Spp	-	1	-	1(5.5%)
Total	12(66.66%)	4(22.22%)	2(11.11%)	18

The table above shows that among the total 18 isolates 12(66.66%) were isolated on the third day, 4 (22.22%)were isolated on the seventh day and 2 (11.11%) were isolated on both third and seventh day.

Majority of the isolates were *E.coli* (44.4%) followed by *Klebsiella .spp*(22.2%), *Proteus .spp* (16.6%), *Enterococci* (5.5%), *Candida. Spp*(5.5%) and *CONS*(5.5%).

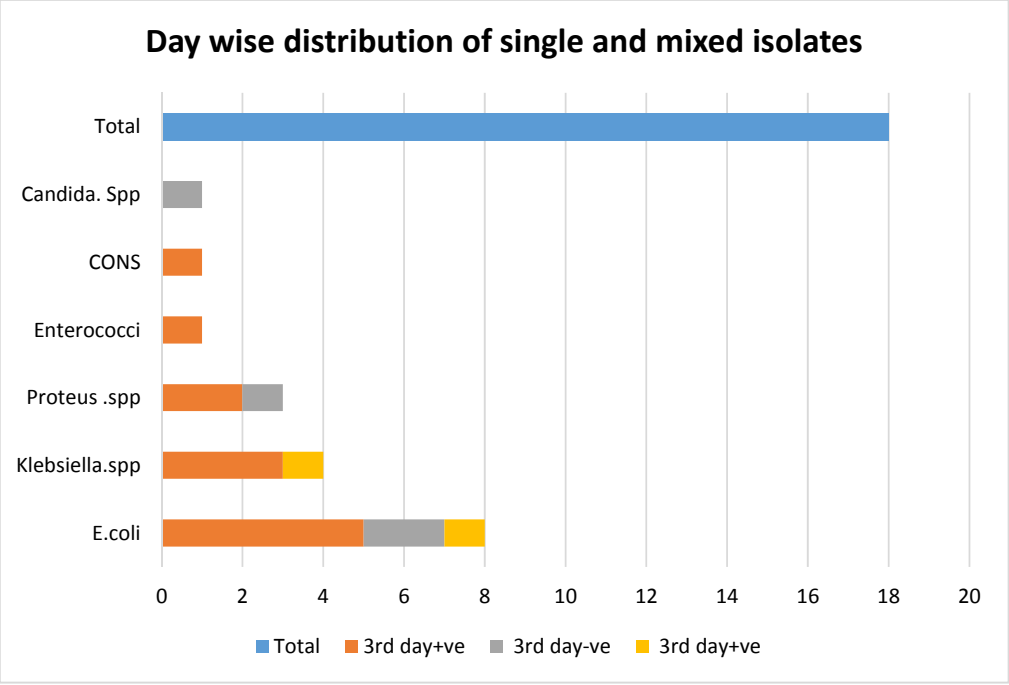


Table -28 Distribution of isolates among different age groups

Isolates	Age group						Total isolate
	21-30	31-40	41-50	51-60	61-70	71-90	
E.coli	-	3	1	3	0	1	8
Klebsiella.spp	-	1	1	-	2	-	4
Proteus .spp	-	-	1	2	-	-	3
Enterococci	-	-	-	1	-	-	1
Candida.spp	-	-	-	1	-	-	1
CONS	-	1	-	-	-	-	1
Total	-	5(27.7%)	3(16.6%)	7(38.8%)	2(11.1%)	1(5.5%)	18

The above table shows tha1t out of 18 isolates majority of isolates were from the 50-60 age group (38.8%) followed by 31-40 age group (27.7 %). Predominant isolates were *E.coli* followed by *Klebsiella .spp*.

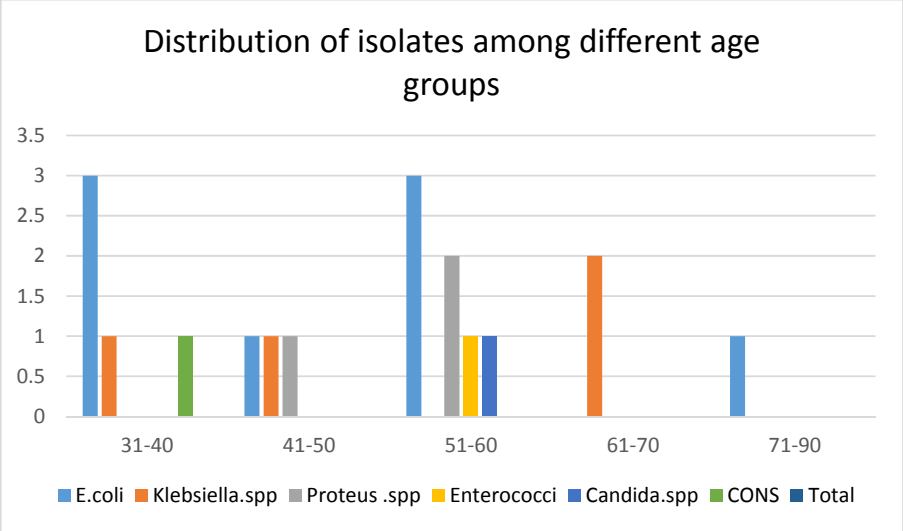


Table-29 Colony counts of isolates

Isolates	Colony count				Total isolates
	$<10^2$	10^2-10^4	10^4- 10^5	$>10^5$	
E.coli	1	3	2	2	8
Klebsiella.spp	-	2	1	1	4
Proteus .spp	-	-	2	1	3
Enterococci	-	1	-	-	1
Candida.spp	1	-	-	-	1
CONS	-	1	-	-	1
Total	2	9	5	4	18

The table above shows that 4 patients (4%) out of 100 patients had significant colony count ($>10^5$ CFU/ml). Majority of the patients had colony counts inbetween 10^2-10^4 CFU/ml (9%).

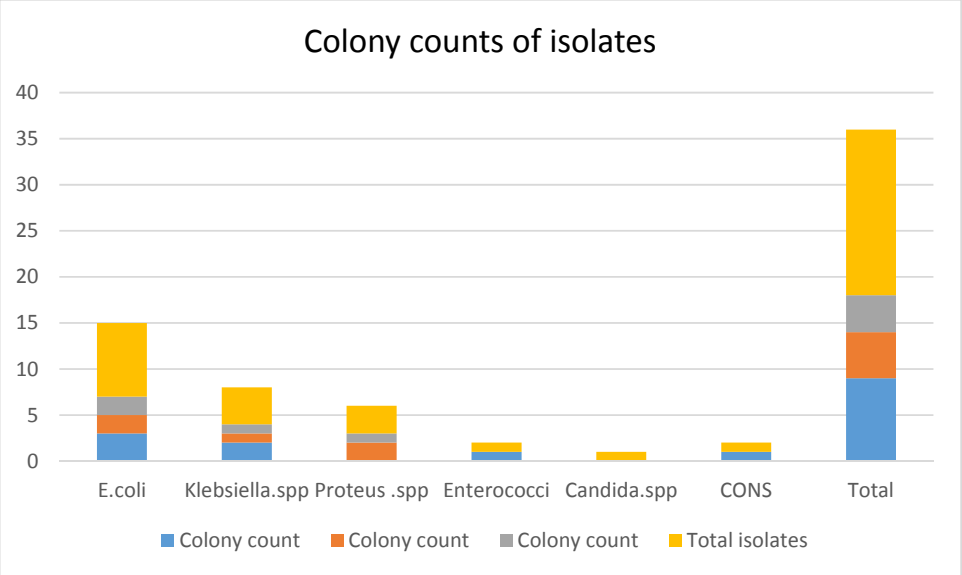


Table-26 Daywise analysis of significant colony count (>10^5cfu/ml)

Isolates	3rd day+ve 7th day-ve	3rd day-ve 7th day+ve	3rd day+ve 7th day+ve	Total
E.coli	1	-	1	2
Proteus .spp	-	1	-	1
Klebsiella.spp	1	-	-	1
Total	2(2%)	1(1%)	1(1%)	4(4%)

Among the 100 patients only 4 (4%) patients had more than 10^5cfu/ml.One patient on the third day and one (1%)patients on both third day and seventh day had significant colony count.

ANTIBIOGRAM OF ISOLATES – URODYNAMIC STUDY

Table-30(A)Bacterial isolates -Gram negative bacilli

Isolates	AK		GM		NF		NX		CTX		AMP	
	S	R	S	R	S	R	S	R	S	R	S	R
8-*E.coli*	8 (100%)	0	2 (25%)	6 (75%)	7 (87.5 %)	1 (12.5%)	7 (87.5%)	1 (12.5%)	7 (87.5%)	1 (12.5%)	3 (37.5%)	5 (62.5%)
4-*Klebsiella.spp*	3 (75%)	1 (25%)	1 (25%)	3 (75%)	4 (100%)	0 (0%)	3 (75%)	1 (25%)	3 (75%)	1 (25%)	1 (25 %)	3 (75%)
3-*Proteus .spp*	3 (100%)	0 (0%)	0 (0 %)	3 (100%)	2 (66.67%)	1 (33.33%)	3 (100%)	0 (0%)	3 (100%)	0 (0%)	0 (0 %)	3 (100%)

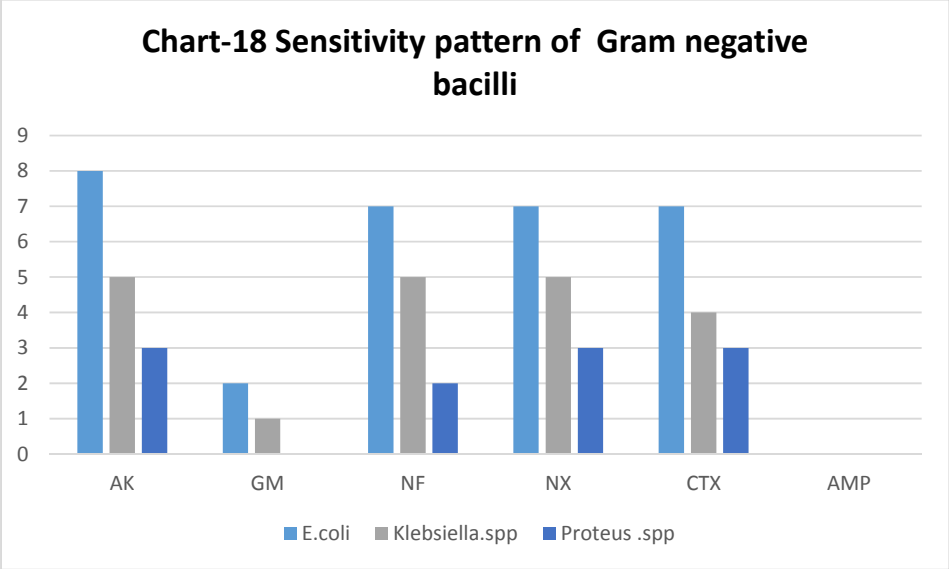


Table-30 (B) Gram positive cocci- *Enterococci. spp*

Isolates	NF		PEN		NX		HLG		CIP		AMPI	
	S	R	S	R	S	R	S	R	S	R	S	R
1-*Entero-cocci*	1 (100%)	0	0	1 (100%)	1 (100%)	0	1 (100%)	0	0	1 (100%)	0	1 (100%)

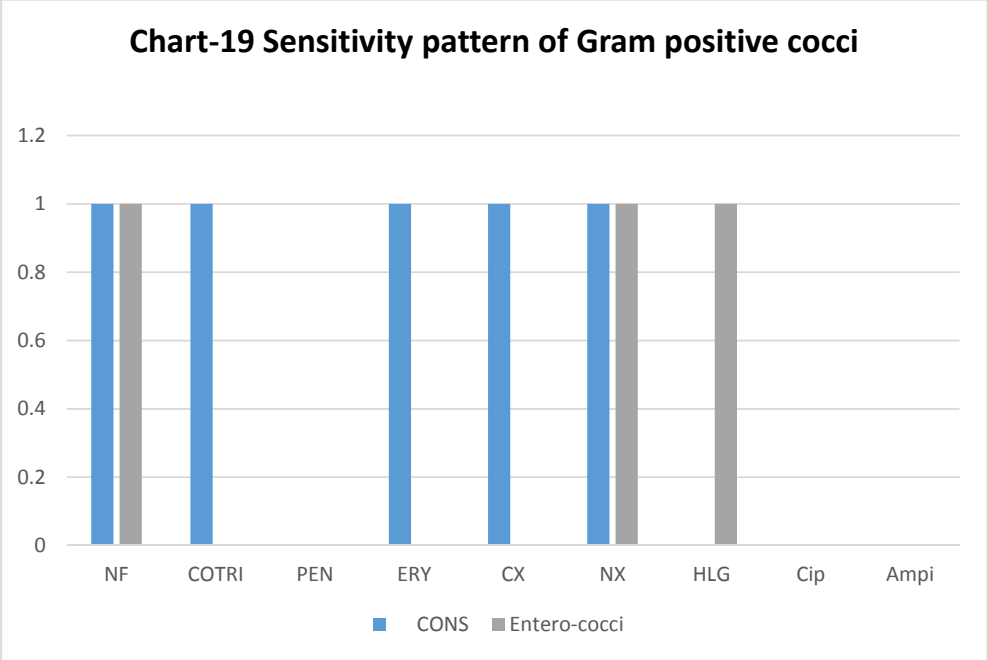


Table-30(C) Fungal isolates

Isolate	Total isolate	Fluconazole		Voriconazole	
		S	R	S	R
Candida albicans	1	1	0	1	0

MIC OF ANTIBIOTICS TESTED

Table-31(A) MIC of Vancomycin to Staphylococcus aureus

Number of isolates tested-1

	Sensitive	Intermediate	Resistant
Antibiotic concentration µg/ml	<2	4-8	8-16
Vancomycin	1	-	-

The isolate tested was within the MIC sensitivity range.

Table-31(B) MIC of Fluconazole and voriconazole to Candida albicansNumber of isolates tested-1

	Fluconazole			Itraconazole			Voriconazole		
	S	SDD	R	S	SDD	R	S	SDD	R
Antibiotic concentration µg/ml	≤8	16-32	≥64	≤0.125	0.25-0.5	≥1	≤1	2	≥4
Candida spp	2	-	-	0.0625			0.25	-	-

The isolate tested was within the MIC sensitivity range.

CYSTOSCOPY AND URODYNAMIC STUDY

Table-31 Gender wise distribution of samples

Procedure	Number of Males	Number of Females	Total patients
Cystoscopy	55	45	100
Urodynamic study	54	46	100
Total	109(54.5%)	89(44.5%)	200

Among 200 patients 109 were from males and 89 were from females.

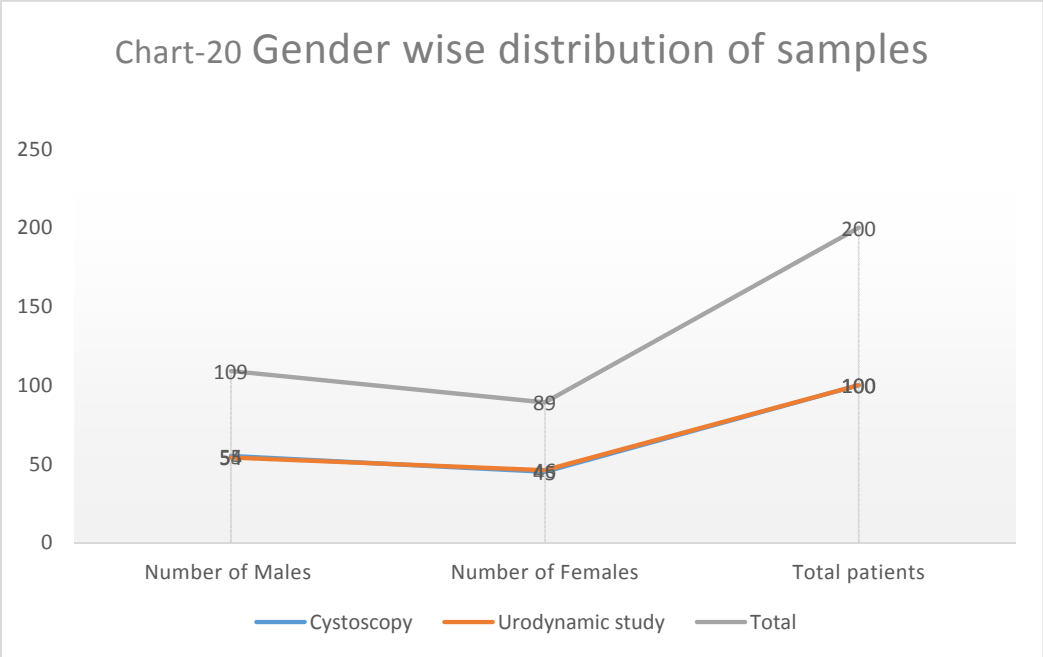


Table-32 Culture positive and negatives

Procedure	Number of Culture +ve patients	Number of Culture -ve patients	Total patients
Cystoscopy	38	62	100
Urodynamic study	15	85	100
Total	53(26.5%)	147(73.5%)	200

53(26.5%) patients were culture positive and 147(73.5%) patients were culture negative.

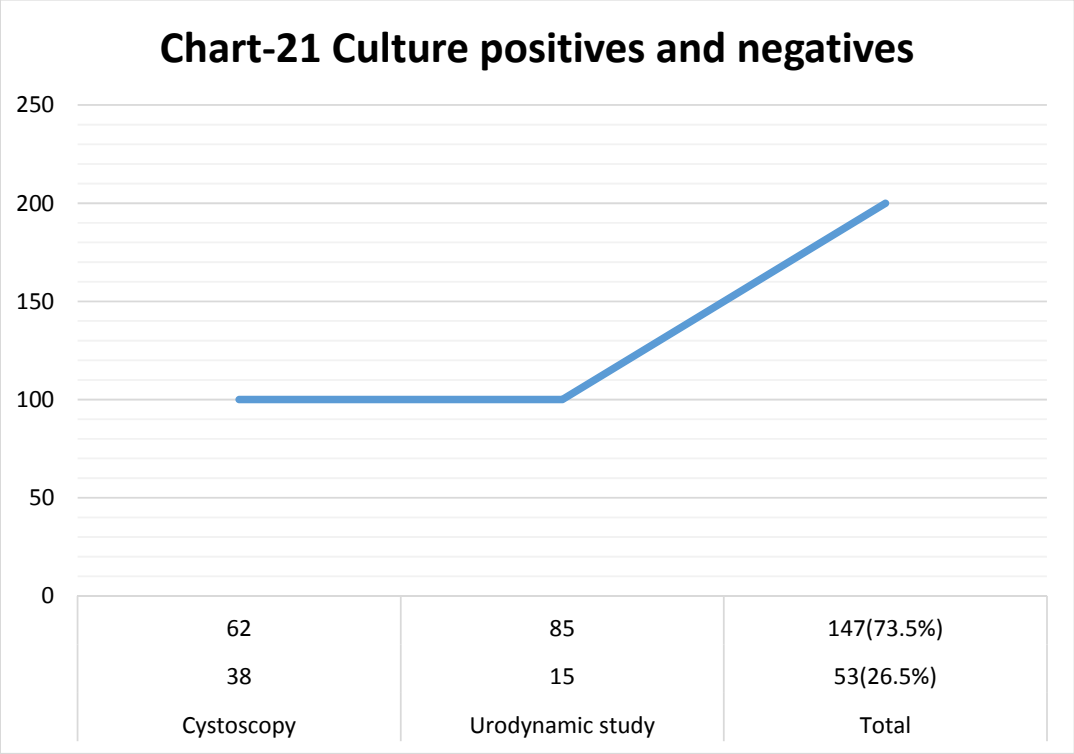


TABLE-33 Single and mixed isolates

Procedure	No of cuture +ve pts with Single isolate	No of cuture +ve pts with Mixed (Two)isolate	Total No of cuture +ve pts
Cystoscopy	33	5	38
Urodynamic study	12	3	15
Total	45(84.9%)	8(15.09%)	53

Among the 53 culture positive 48 patients had single isolates and 8 patients had mixed isolates.

Table-34 Distribution of isolates

Isolates	Cystoscopy	Urodynamic study	Total
E.coli	17	8	25 (40.9%)
Klebsiella.spp	6	4	10 (16.3%)
Proteus .spp	6	3	9 (14.06%)
Enterococci	4	1	5 (7.81%)
Acinetobacter.spp	3	0	3 (4.68%)
Candida.albicans	2	1	3 (4.68%)
Candida.tropicalis	1	0	1 (1.56%)
Pseudomonas aeruginosa	2	0	2 (3.12%)
CONS	1	1	2 (3.12%)
Staph.aureus	1	0	2 (3.12%)
Total	43(70.49%)	18(29.5%)	61

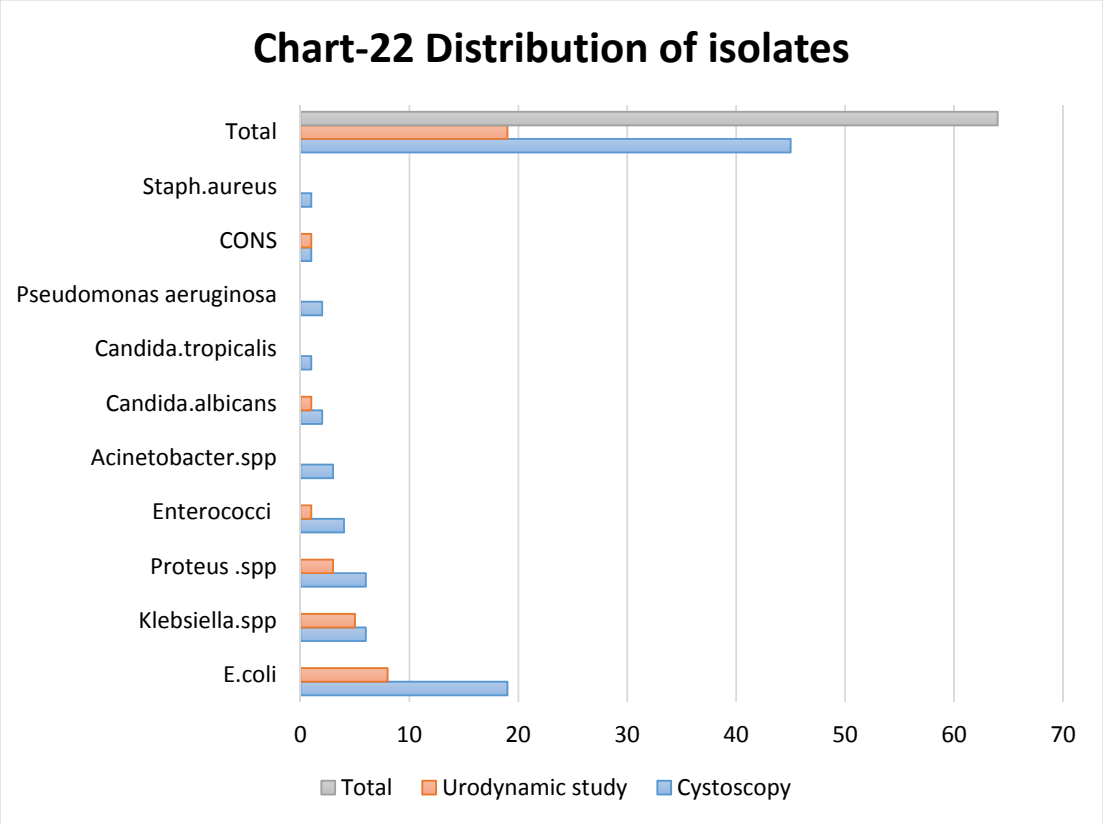


Table-35 No. of pts with Significant colony count (>10^5 CFU/ml)

Procedure	No of patients	*E.coli*	*Proteus spp*	*Klebsiella.spp*	Total%
Cystoscopy	100	3	2	-	5%
Urodynamic study	100	2	1	1	4%
Total	200	5	3	1	4.5%

In cystoscopic study 5(5%) patients had significant colony count.In urodynamic study 4(4%) patients had significant colony count.

Table-36 ESBL producers

Procedure	*E.coli [25]*	*Klebsiellaspp[10]*	*Proteus spp[9]*
Cystoscopy	2	1	1
Urodynamic study	1	1	-
Total	3(12%)	2(20%)	1(11.11%)

TABLE-37 omp T GENE IN *E.coli*isolates with >10 ^{5} CFU/ml

GENE	PRIMER SEQUENCES	AMPLICON SIZE IN BASE PAIRS	RESULT
ompT	5'-CACGCTCCACAAACCAAGTG-3	**1120**	**Negative**

Bright band was not seen on 1120 base region of ompT gene.

Colour Plates

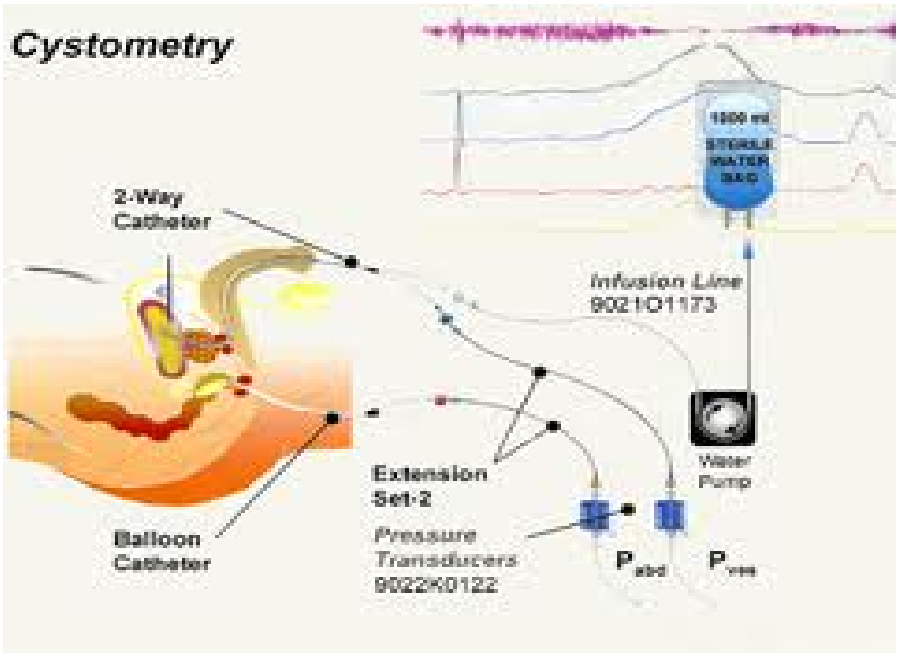
FIGURE-1 CYTOSCOPE

RIGID CYSTOSCOPE **FLEXIBLE CYSTOSCOPE**

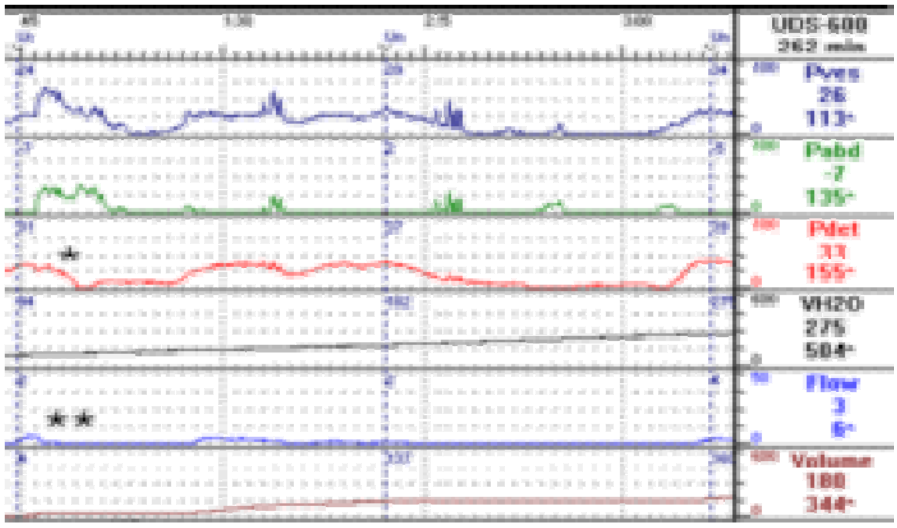
FIGURE-2- URODYNAMICS

URODYNAMIC MACHINE

URODYNAMICS- CETHETERS FOR PRESSURE MEASUREMENTS



URODYNAMICS- SHOWING PRESSURE PATTERN



* Detrusor Instability

** Leak

FIGURE- GRAM STAIN OF UNCENTRIFUGED URINE SAMPE SHOWING ONLY PUS CELLS

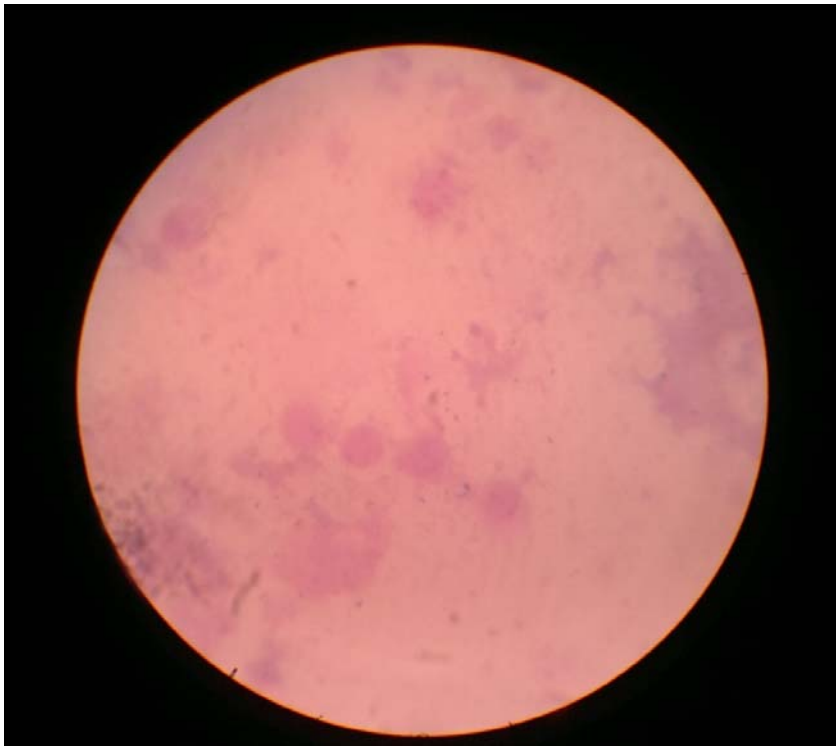
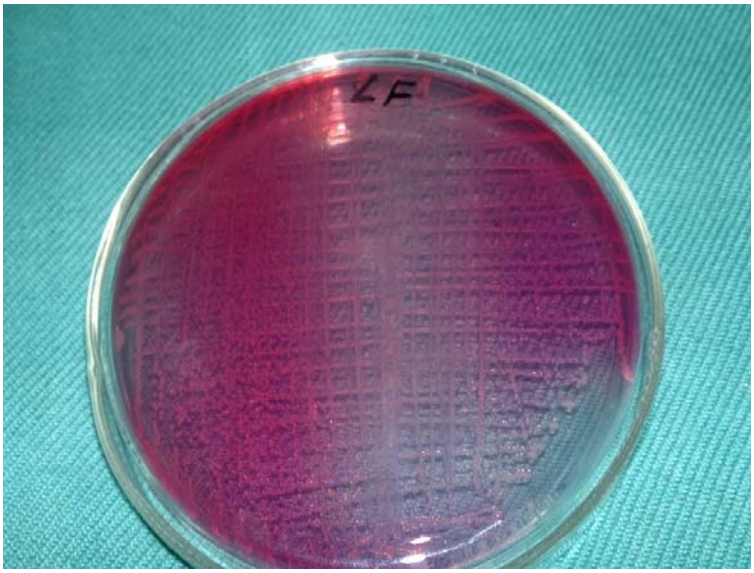


FIGURE-3 SEMIQUANTITATIVE CULTURE METHOD



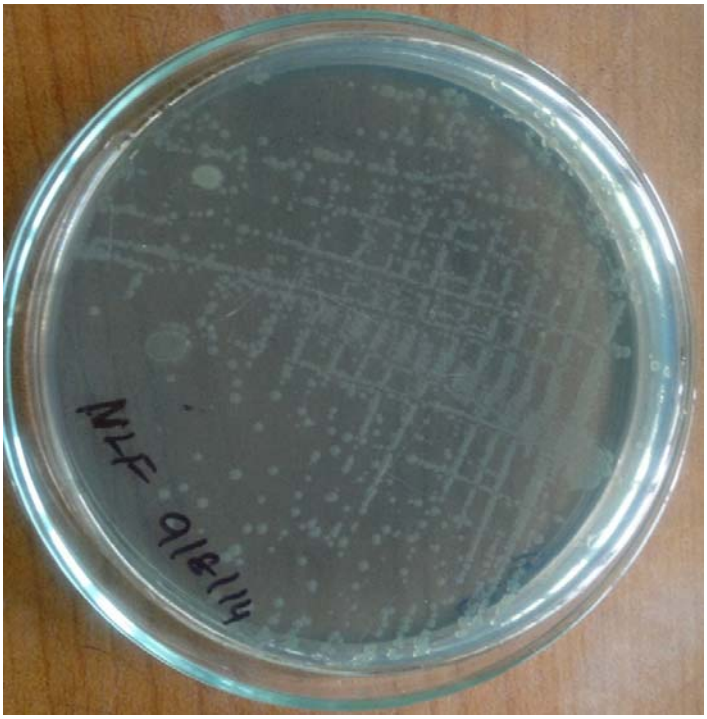


FIGURE-4 SEMI QUANTITATIVE CULTURE METHOD – ENTEROCOCCI

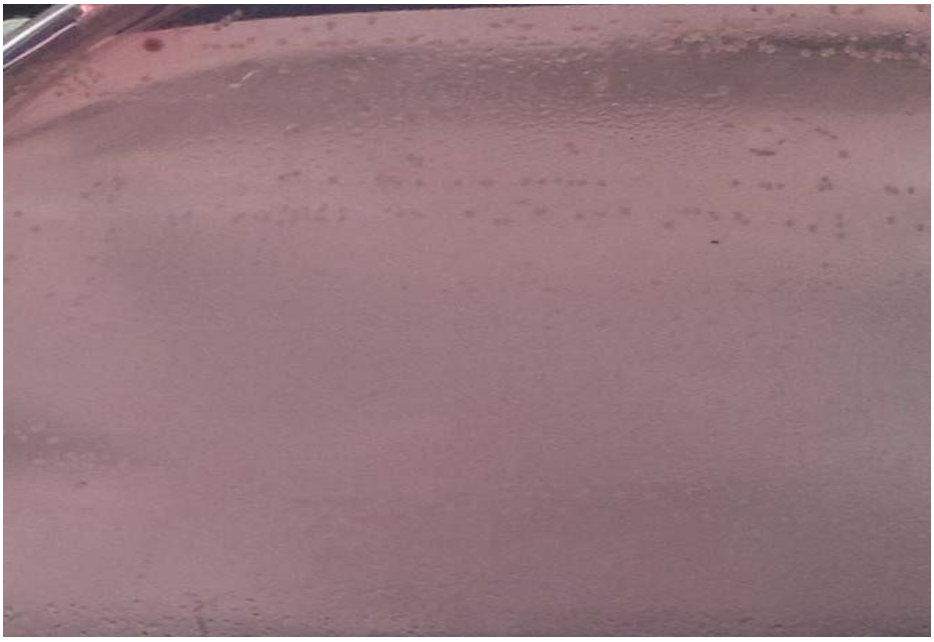


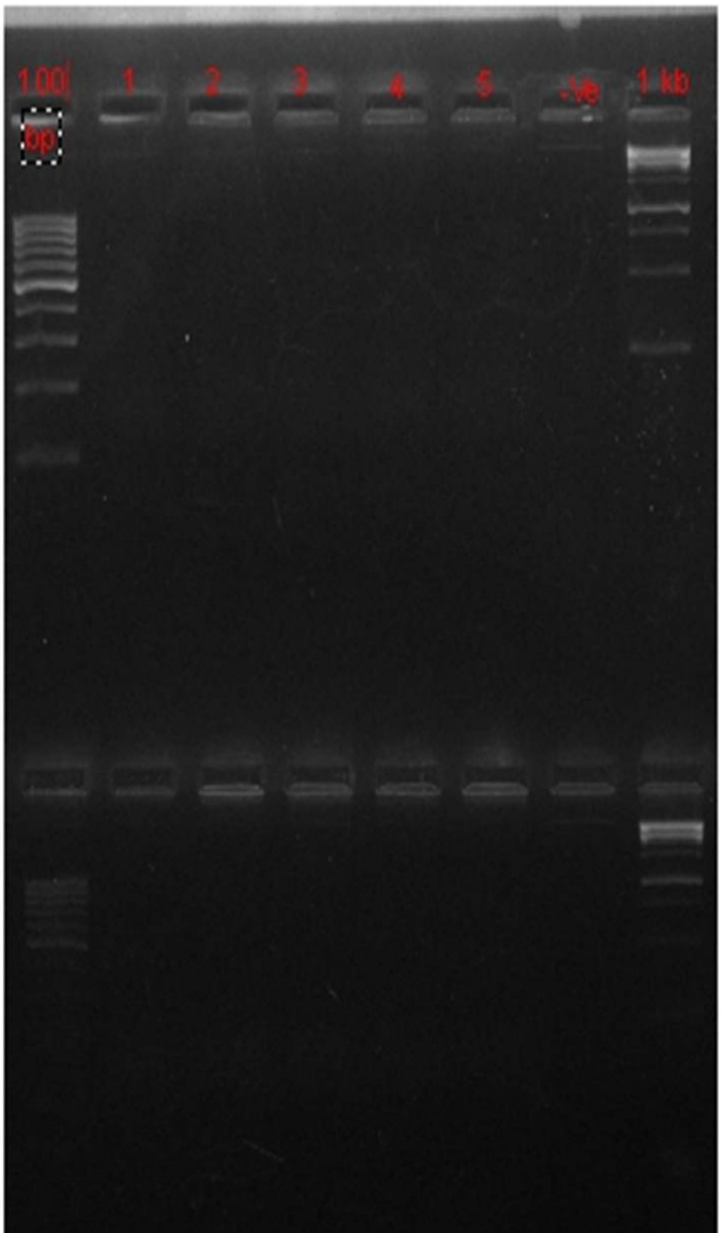
FIGURE-5 ANTIBIOGRAM- GRAM NEGATIVE BACILLI-I LINE DRUGS

FIGURE-6 PHENOTYPIC CONFIRMATION DISC DIFFUSION TEST FOR ESBL PRODUCTION

FIGURE-7 MINIMUM INHIBITORY CONCENTRATION OF STAPHYLOCOCCUS AUREUS BY MACRO BROTH DILUTION METHOD – 1 µg/ml

FIGURE-8 DETECTION ompT GENE IN *E.coli* by conventional PCR

BAND WAS NOT SEEN ON THE 1120 Kb REGION



ompTgene band region -1120 Bp

FIGURE-9 C.*albicans* and C.*tropicalis* on CHROM agar

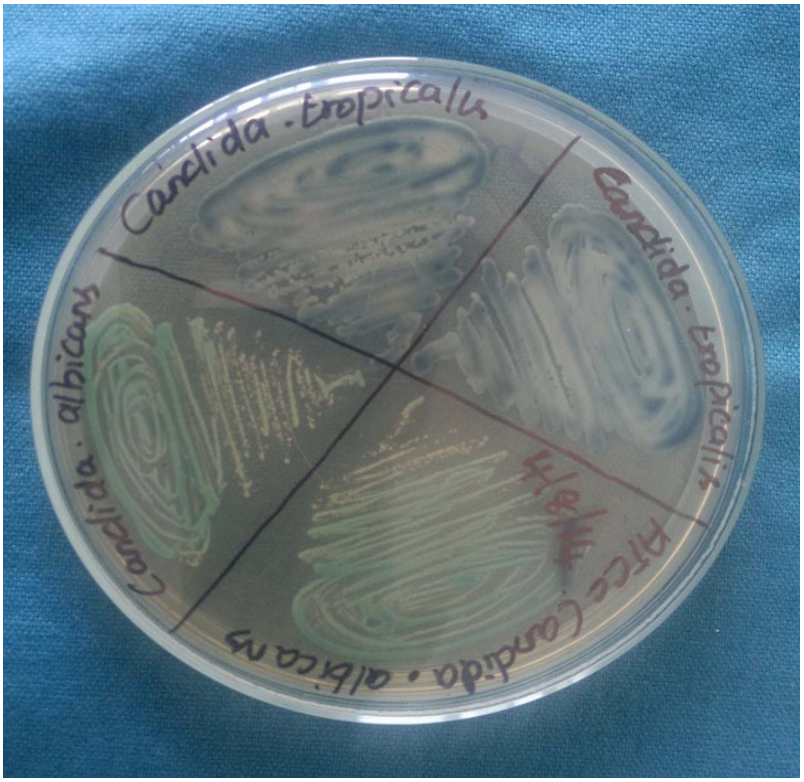


FIGURE-10CORN MEAL AGAR – C.tropicalis showing small clusters of blastoconidia along the long pseudohyphae

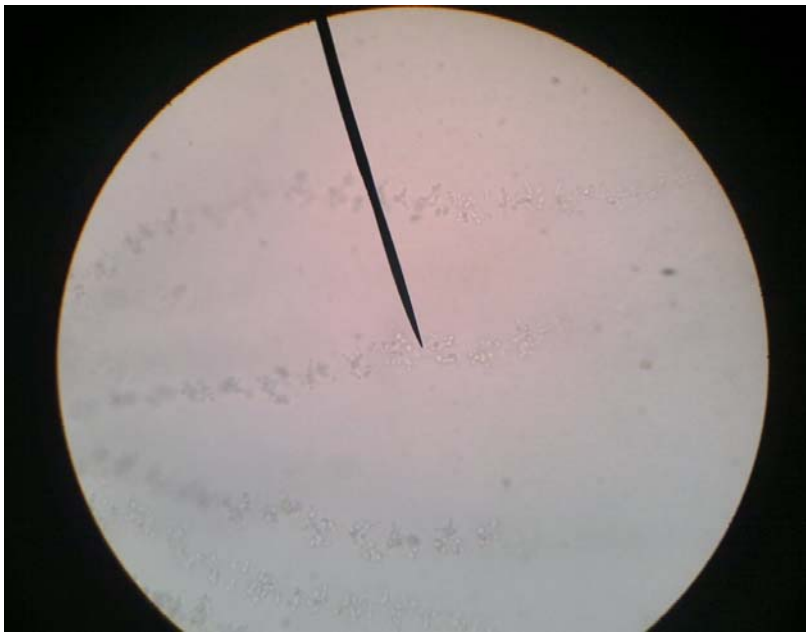


FIGURE- 11 SUGAR FERMENTATION

***C.tropicalis* – Fermentation of maltose, sucrose and glucose**

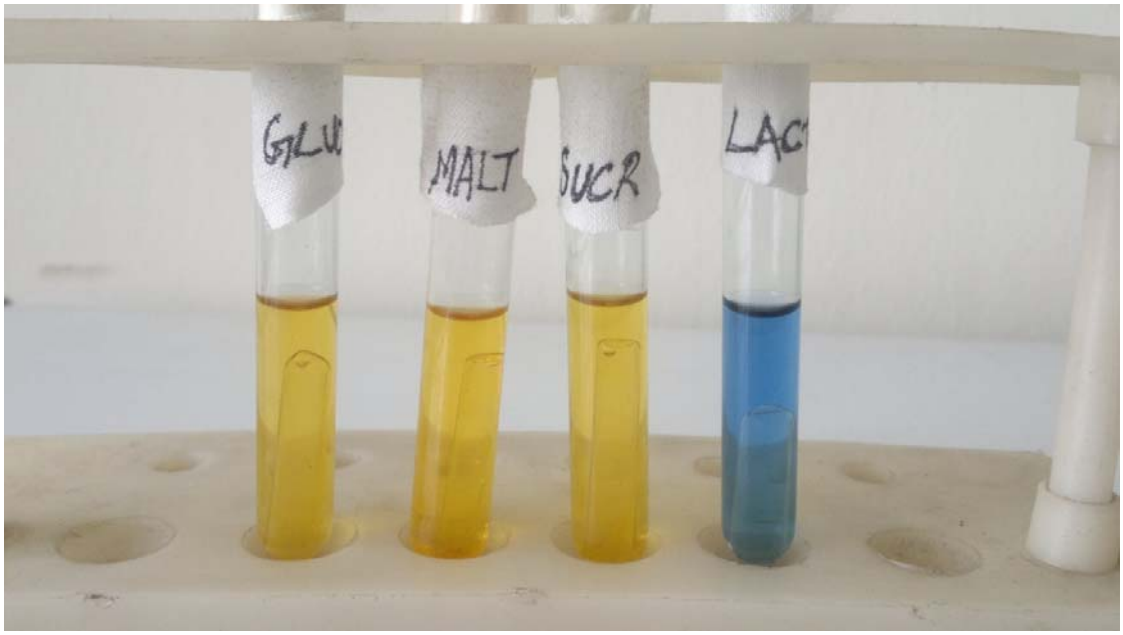


FIGURE- 12 SUGAR ASSIMILATION OF GLUCOSE, TREHALOSE AND SUCROSE-*C.albicans*

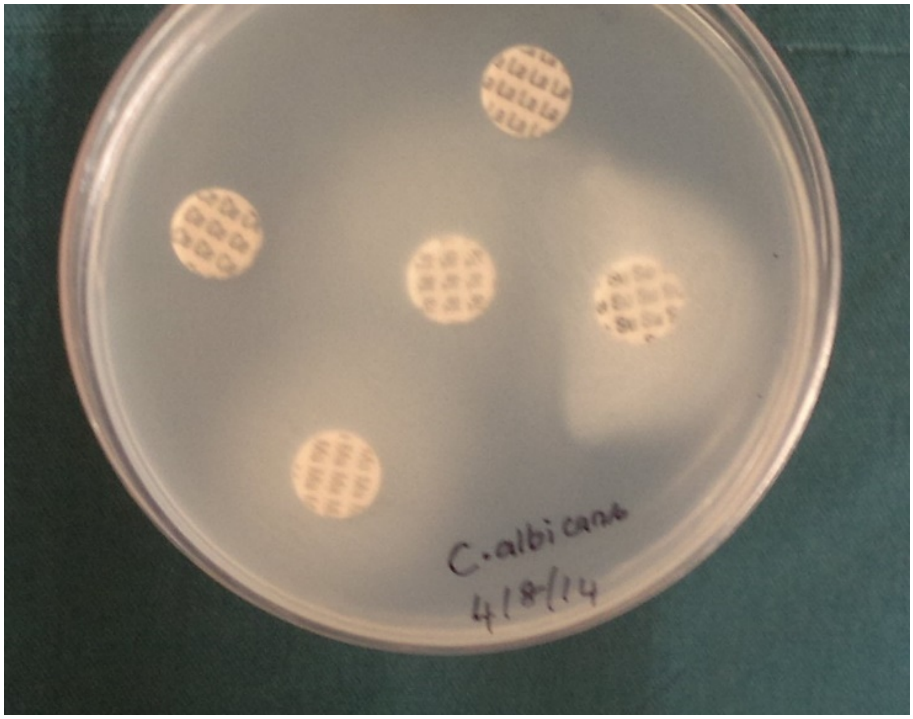


FIGURE-13 SENSITIVITY PATTERN OF ATCC and test isolate of *C.albicans*

Sensitive-Flucanazole-20mm, Voricanazole- >17mm

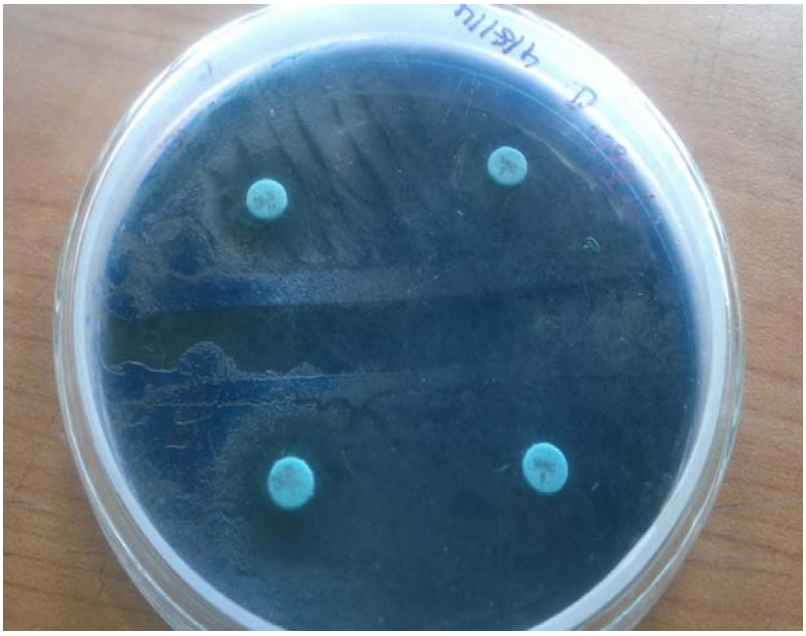
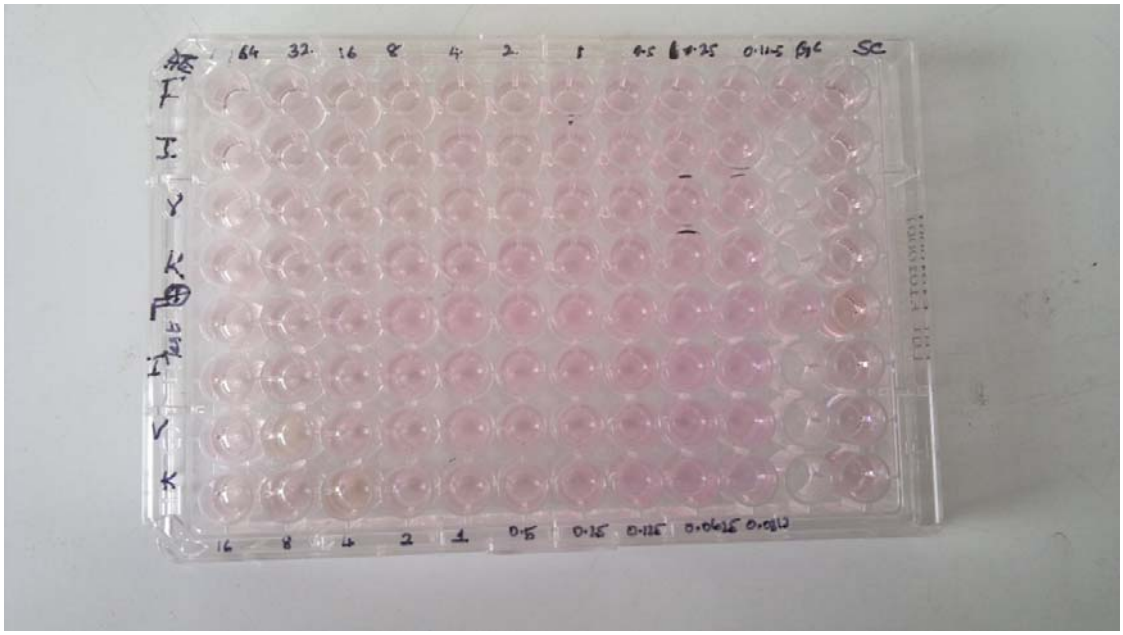


FIGURE- 14 MIC OF Flucanazole- 2 µg/ml, Itraconazole- 0.0625 µg/ml and Voricanazole µg/ml -2-*C.albicans*



Discussion

DISCUSSION

Various OP diagnostic, therapeutic procedures were carried out in our urology department, RGGGH. Of which cystoscopy and urodynamic procedures were commonly employed for various disease conditions. Nearly 250 cases in cystoscopy every month and 15 cases every month were carried out in urology.

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, therapeutic purposes or both. Manipulation of the urinary tract can result in significant injury. The patient should understand the proposed procedure and potential complications. A patient who is comfortable, informed, and assured will more likely cooperate and tolerate the procedure. [53]

Cystoscopic examinations are performed extensively for the diagnosis and management of various urological disorders.Urodynamic studies were extremely valuable in devising a therapy based on altered physiology of the lower urinary tract. However these invasive investigations may be associated with marked morbidity.The morbidity of these procedures has been studied only in the context of urinary tract infections.

Urinary tract infection is the most common nosocomial infection, comprising about 35 % of such occurrences in both hospitals and nursing homes. In both types of institutions, UTI_S are usually associated with devices intended to assist in the drainage of urine. In hospitals were the epidemiology has been investigated, 80 % or more of the nosocomial UTI_S are related to the use of urethral catheters. Another 5 % to 10 % occur after genitourinary manipulations [54]. As a predisposing factorurological invasive proceduresaccount for up to 10% of the hospital acquired infections [55].

In the present study 600 urine samples were collected from 200 patients attending Cystoscopy and Urodynamic study at Urology department, RGGGH, Chennai.

In theCystoscopic study conducted among 100 Patients 38 patients were culture positive 62 were patients were culture negative. Among these 38 culture positives 21 (38.18%) were males and 17 (37.77%) were females. There was no significant difference in culture positives among male and females. Among the 21 culture positive males > 40 age group had higher culture positives (>37 %) than < 40 age groups (25%). Increased instrumentation, and prostatic hypertrophy account for bacteriuria in older age group in males [56,57].

Among 17 culture positive females extremes of age groups 18-30 (100%) and 71-90 (50%) had higher culture positives than the age groups in between but the number of patients were very limited so more number of patients were needed to substantiate these results. During adolescence, the incidence of UTI significantly increases to 20% in young women, while remaining constant in young men (Sanford,1975) [56]. Increased instrumentation and incontinence account for bacteriuria in older age group in females [56,57].

In the Urodynamic study conducted among 100 Patients 15 patients were culture positive and 85 patients were culture negative. Among the 15 culture positive patients 8 (14.8%) were males and 7 (15.2%) were females. Similar to cystoscopic results there was no significant difference in culture positives among male and females. Among the 18-30 age group there were no culture positives in males but 50 % were culture positives in females.

In Cystoscopy study conducted the older age group (>40) females were less culture positives (<15%) compared to younger age (<40) groups (>25%). Prevalence of UTI increases with age in females [58]. Although Harrari*et al* found that bacteriuria incidence higher in elderly, the difference was not satistically significant.[59] Association of acquiring infection with increasing age which was found before urodynamic study was not seen after post

urodynamic study. This finding is in agreement with others who found no significant association between advancing age and UTI after urodynamics.

Wet mount and Gram stained preparations were used for microscopy. Wet mount of uncentrifuged urine was done usually to find out the presence of pus cells, epithelial cells and microorganisms. Pyuria is highly sensitive indicator of UTI [60] but some considers it as poor predictor of infection [62]. It indicates only inflammation not always infection [61]. In our present study though all the samples showed pus cells only 40 (13.33%) samples and 16(5.33%) samples were culture positive from 300 samples each of cystoscopy and urodynamic study respectively. So presence of pus cells may not be the reliable indicator of UTI.

In post cystoscopy procedure study 9 (22.5%) samples out of 40 samples and 5 (31.25%) samples out of 16 samples from cystoscopy and urodynamic study respectively which were showing bacteria on Grams stain smear also showed growth on culture with 5 samples and 2 samples having significant colony count from cystoscopy and urodynamic study respectively . In 31(77.5%) and 11(68.75%) no bacteria was seen on Gram stain smear in cystoscopy and urodynamic study respectively. They also showed growth on culture revealing low sensitivity of Gram stain compared to culture.

More than 90% of microscopic bacteriuria was detected in uncentrifuged Gram stained urine with colony counts 10 ^{5}CFU/ml [63]. Others studies indicate that Gram stain is of low sensitivity in detecting UTI [64].

In the cystoscopic study 43 isolates were grown from the 38 culture positive patients. Out of38 patients 33 patients yielded single isolate on culture and five patients yielded two isolates on culture.Most of the patients were culture positive on third day (28 out of 38) and they turn out to be culture negative on seventh day. 8 patients who were culture negative on third day were culture positive on seventh day. Third day culture positive samples turned out to be culture negative on seventh day indicating acute transient infection .Since it disappeared on the seventh day antibiotics was unnecessary. Third day culture negative samples turned out to be culture positive on seventh day may due unhygienic practices and since they are asymptomatic antibiotics need not to be administered and requested to give samples on tenth day.

And 2 (2%) patients out of 100 patients were culture positive on both third and seventh day with significant colony count $>10^5$ CFU/ml and one patient was symptomatic indicating significant persistent infection of these patients and they had underlying conditions such as diabetes and this symptomatic patient was treated with first line antibiotics . With a cut-off level of $>10^5$ CFU/ml only 5 isolates could be classified as significant bacteriuria. Out of 100 patients only 5 patients (5%) had significant colony

count >10 ^{5}CFU/ml. Cystoscopy performed in the outpatient setting carries a risk of post-procedure UTI of between 2.7% and 4.5% [65]. This finding was similar to our study.

In our study, the most predominant urinary pathogen isolated from the post cystoscopy patients was *E. coli (39.5%)*. This finding was similar to previous observations [66,67,74]. The other predominant pathogens were *Klebsiellaspp*(13.9%) and *Proteus spp*(13.9%).Other isolates were *Acinetobacterspp*(6.9%), *Pseudomonas aeruginosa*(4.6%), *Enterococci* (9.3%),*S.aureus*(2.32%), *CONS* (2.32%*) and Candida spp*(6.9%).

In the post procedure urodynamic study conducted, 18 isolates were grown from the 15 culture positive patients. Among these 15 patients 12 (12%) yielded single isolate on culture and 3(3%) patients yielded two isolates on culture. Most of the patients were culture positive on third day (10 out of 15) and they turn out to be culture negative on seventh 3 patients who were culture negative on third day were culture positive on seventh day. Third day culture positive samples turned out to be culture negative on seventh day indicating acute transient infection. Since it disappeared on the seventh day antibiotics was unnecessary Culture positive only on seventh day may be due to improper perineal toileting.Since they are asymptomatic antibiotics need not to be administered and requested to give samples on tenth day.

And 2 (2%) patients were culture positive on both third and seventh day with significant colony count $>10^5$ CFU/ml indicating significant persistent infection. On post-test sampling 18 cultures were positive. With a cut-off level of $>10^5$ CFU/ml only 4 isolates could be classified as significant bacteriuria. Only 1 was symptomatic and this patient had diagnosed as neurogenic bladder. The remaining had no symptoms following the post urodynamic study. Thus the incidence of lower urinary tract infection was 4 % following diagnostic catheterization. We concluded that although bacteriuria is common after diagnostic catheterization, it is commonly asymptomatic. Out of 100 patients only 4(4%) patients had significant colony count $>10^5$CFU/ml in our study but higher rates of infection (7.5%) after urodynamic procedure were reported by Bergeman*et al* [68]. We have 5% of significant counts in post-cystoscopy and 4% significant count in post-urodynamic study similar to the study by Allahmella et al.[78]We should also note that 5 patients from Urodynamic and 9 patients from cystoscopy had counts inbetween 10^4-10^5CFU/ml.

In this study, the most predominant pathogen isolated from the post urodynamicswas*E.coli* (44.4%). The second predominant pathogen isolated in our study was *Klebsiellaspp*(22.2%) These finding were similar to previous observations [69]. Other isolates were *Proteus spp*(16.6%), *Enterococci (5.5%)*, *CONS (5.5%)* and *Candida spp(5.5%).* One member of *Candida spp (Candida albicans)*was isolated in our study. *S. aureus*was not isolated in our study.

*E. coli, Proteus spp*and *Enterococci spp* have been reported by Leblebicioglu et al[70] as the most frequently acquired bacterial pathogens .

Of the 25*E. coli* isolates tested in our post procedure study 58.8 % were resistant to ampicillin, 4 % were resistant to Amikacin, 64 % were resistant to Gentamycin, 20 % were resistant to Nitrofurantoin, 12 % were resistant to Norfloxacin and 12 % were resistant to Cefatoxime. Among these 3 were ESBL producers (12%). It showed low resistance to nitrofurantoin (20 %) may be due to less frequent prescription of these drugs and these reports were similar to reports by Biswas *et al and* Kauser *et al* [71,72]. Low level of resistance of Amikacin was observed in our study compared to other studies. But it showed high level of resistance to gentamycin in our study. In India aminoglycoside resistance ranges between 5[72] % to 36.1 % [73]. Low level of resistance of Norfloxacin was observed in our study similar to reports by Arjunan*et al* [75].

There are many virulent genes associated with uropathogenic*E. coli*. We have tested one of the virulent gene ompT(outer membrane protein) for the *E. coli*whether it is present in patients with significant colony count and symptoms.Of the 25 *E. coli*isolates 5 isolates had colony count of 10 5 CFU/ml were tested for the presence of ompT gene by conventional PCR method. None of these isolates showed positive band for the ompT gene.

E. coli isolates obtained after bladder instrumentaion usually lacks virulence factors was reported by Zdziarski et al similar to our results. Asymptomatic bacteriuria appear to be lose their ability to express functional virulence associated genes.

The common pathogen isolated next to *E. coli was Klebsiellaspp*in our study and it is the same in most of the Indian studies. All the 10 isolates exhibit high level of resistance to ampicillin (80%) and gentamycin (40%) compared to other drugs as in other studies [71.76]. It showed low level of resistance to amikacin (20%), nitrofurantoin (10%), norfloxacin (20%) and cefotaxime (20%) compared to other studies [71,76]. Among the 10 isolates 2(20%) were ESBLproducers.

Of the 9 *Proteus spp* isolates all are sensitive to amikacin whereas 55.55% of the isolates showed resistance to gentamycin. It showed high resistance to ampicillin (77.77%). Resistance pattern of other drugs were cefotaxime (11.11%), nitrofurantoin (33.33%) and norfloxacin (11.11%). Among the 9 proteus isolates 1 was a ESBL producer.

Of the non *Enterobactericeae*group 2 *Pseudomonas aeruginosa*and 3 *Acinetobacterspp*were isolated in our postcystoscopy study and not in urodynamic study. *Pseudomonas aeruginosa*showed 100 % resistance to ciprofloxacin similar to the study by Bhargavi et al [77]. These 2 isolates were

sensitive to amikacin, norfloxacin, ceftazidime and piperacillintazobactum. High resistance were observed for these drugs in other studies[76,77]. Three *Acinetobacterspp*were isolated in our study with 33.33% resistance to gentamycin and ciprofloxacin. They were all sensitive to amikacin, norfloxacin, ceftazidime and piperacillintazobactum.

Enterococci spp constitutes 7.8% of the total isolates and it was the fourth commonest pathogen isolated in our study. Four were *E. faecium and 1 was E. faecalis.* It showed 80% resistance to penicillin and ciprofloxacin , 60% resistance to ampicillin and 40 % resistance to nitrofurantoin and norfloxacin. 28.57% resitance was reported for norfloxacin in other study [77]. The resistant pattern for nitrofurantion was similar to Mahesh et al study [77].

One isolate of *CONS* (3.1%) and two isolates of *S. aureus* (1.5%) were isolated in our study. They were all resistant to penicillin (100%) and sensitive to all other drugs tested except the *S.aureus* was methicillin resistant. MIC values of vancomycin to *S.aureus* was within the sensitivity range.

Candiduria is an increasingly common finding in hospitalized patients and most of the patients were asymptomatic as there are no associated signs and symptoms. Four isolates of Candida spp (6.25%) were isolated in our study. Three were *C. albicans*and 1 was *C.tropicalis*in our study and they were all sensitive to fluconazole and voriconazole and their MIC values were within

their sensitivity range.*C.tropicalis* were second most commonly isolated next to *C. albicans*. All these isolates had insignificant colony counts and were from female patients having Diabetes. Comorbid conditions associated with candiduria were surgical procedures, diabetes mellitus, urologic abnormality, female sex, invasive genitourinary devices and antimicrobial use.

Summary

SUMMARY

A total of 100 patients each undergoing for cystoscopy and urodynamic study were evaluated for UTI. Three samples were collected from each patient. The samples which were culture positive on the day of the procedure were subsequently excluded from the study.

Culture positivity rate is marginally higher in males than females. In cystoscopy culture positivity rate was higher in the above 40 age groups and in urodynamics extremes of age group had higher culture positivity rate.

Cultures of samples were considered the reliable method when compared to wet mount and Gram stain. *E.coli* was the commonest pathogen and second commonest pathogen was *Klebsiella.spp* isolated in our post procedure study similar to other studies. Others were *Enterococci spp, Proteus spp, P.aeruginosa, Acinetobacterspp, CONS*and*S.aureus .Candida.Spp*was isolated with insignificant colony counts.

Semiquantitative method was employed to detect colony counts. With a cut off level $>10^5$count as significant colony count only five patients from post cystoscopy and our from post urodynamic procedure hadsignificant colony counts and only two patients were symptomatic. So the incidence of UTI was

5% and 4% for post cystoscopy and post urodynamic procedure respectively. Others had transient asymptomatic bacteriuria.

The commonest isolate *E. coli*with significant count was not associated with the virulent ompT gene one of the markers for urosepsis.

All isolates exhibited high level resistance to ampicillin. Five ESBL producers were identified. No multiple drugs resistant isolates was detected in our study.

Conclusion

CONCLUSION

Both cystoscopy and urodynamic study are invasive procedures since they are done by instrumentation and catheterization respectively and they are considered as a predisposing factor for UTI in patients undergoing these procedures.

Semi quantitative culture is a reliable method for detecting asymptomatic bacteriuria and defining urinary tract infections. The predominant isolates in post cystoscopy and urodynamic studies were *E.coli* and *Klebsiella spp.*

There is statistical correlation that the rate of infection in post cystoscopy and urodynamic study is marginally higher in the males than the females (P value < 0.05) Out of 200 patients significant persistent infection was seen in two patients and it was associated with other underlying conditions like diabetes and neurogenic bladder. Statistical association of underlying conditions with infection rate was found to be significant (P value < 0.05). No association of virulent gene ompT with *E. Coli*with significant counts.

Both Urodynamic and cystoscopy are safe and well tolerated OP diagnostic procedures, though there is a minimal percentage of transient infection and unnecessary antibiotics need not to be administered.

Appendix

APPENDIX – I

ABBREVIATIONS

ATCC	-	American Type Culture Collections
ESBL	-	Extended spectrum Beta Lactamases
CFU	-	Colony Forming Units
CLSI	-	Clinical & Laboratory Standards Institute
CNF1	-	Colonizing Necrotising Factor
DMSO	-	Dimethyl Sulfoxide
MIC	-	Minimum Inhibitory Concentration
OmpT	-	Outer Membrane Protein
RPMI	-	Rose Well Park Memorial Institute
SDA	-	Sabouraud's Dextrose Agar
PCR	-	Polymerase Chain Reaction
PVR	-	Post Void Residual Volume
PBS	-	Phosphate Buffer Solution
UTI	-	Urinary Tract Infection

APPENDIX -II

A. MEDIA USED

1. CLED Agar

Peptone	4g
Tryptone	4g
Meat extract powder	3g
L-Cystine	0.128g
Distilled Water	1 Ltr
Agar	15g
Bromothymol blue	0.2g
10% Lactose Solution	100ml

Suspend the ingredients in the water, bring to the boil to dissolve, sterilize at 121˚C for 15 minutes and mix well before pouring.

2.Mueller- Hinton Agar

Beef Infusion	300ml
CaseinHydrolysate	17.5g
Starch	1.5g
Agar	Log
Distilled Water	Lltr

Ph = 7.4

Sterilise By Autoclaving At 121°C For 20 Mins

3.Sabouraud Dextrose Agar with Antibiotics

Composition of Sabouraud Dextrose Agar

Dextrose : 20 gm

Peptone : 10 gm

Agar : 20 gm

Distilled Water : 1000 ml

Final pH : 6.9

The ingredients are dissolved by boiling.Chloramphenicol(50mg/lit) and Cycloheximide(500mg/lit) was added .Chloramphenicol was dissolved in 10 ml of 95% ethanol and added to boiling medium. Cycloheximide was dissolved in 10 ml of acetone and added to the boiling medium. Autoclave at 121°C for 15 minutes, Dispense in sterile tubes and allow to cool in slanted position.

4.CORNMEAL AGAR MEDIUM:

Cornmeal : 8gm

Agar : 4gm

Tween 80(1%): 2ml

Distilled water : 200ml

Heat cornmeal and water at 60ºC for 1 hour and filter through filter paper.Add distilled water to make it 200ml and then add agar.Tween 80 is then added.Autoclave it at 121ºC for 15 mins.

5.YEAST NITROGEN BASE MEDIUM

Part A :Agar 40gms/lit

Part B :Yeast nitrogen base 6.7gms/lit

40 grams of part A media is suspended in 900 ml of distilled water. Heat to boiling to dissolve the medium completely.Autoclave at 121º C for 12 minutes.Cool to 50° C and mixed with sterile part B solution aseptically.

6.CHROMAGAR CANDIDA MEDIUM:

Ingredients	Gms/L
Peptone	15.00

Yeast extract	4.00
Dipotassium hydrogen phosphate	1.00
Chromogenic mixture	7.22
Chloramphenicol	0.50
Agar	15.00

Final pH: 6.3.

42.72 grams of media is suspended in 1000 ml of distilled water.Heat to boiling to dissolve the medium completely. Donot autoclave. Cool to 50° C and pour in sterile petridish.

7.MUELLER HINTON AGAR :

Ingredients	**Gms / Litre**
Beef extract	3.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000

Final pH (at 25°C) 7.3±0.2

38grams of media is suspended in 1000 ml of distilled water. Add 20gm of glucose(2%) and methylene blue(0.5µg/ml) is added.Dissolve themedium completely. Dispense and sterilize by autoclaving at 115-121°C for 10 minutes. DO NOT OVERHEAT.

8.**RPMI 1640 MEDIUM[With glutamine and without bicarbonate]**

Obtained commercialy as a dehydrated powder.Suspend 8.4gms of media in 900ml of sterile distilled water.Stirit to completely dissolve the medium donotheat.Sterilize the medium by filtration.Final pH=7.0.

B. MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION

1.Oxidase Reagent

Tetra Methyl P-PhenyleneDiamineDihyrochloride- 1% Aqueous Solution.

2.Catalase

3% Hydrogen Peroxide

3.Kovac's Reagent

Amyl OrIsoamyl Alcohol 150ml Para Dimethyl Amino Benzaldehyde Log

Concentrated Hydrochloric Acid 50ml

Dissolve The Aldehyde In The Alcohol And Slowly Add The Acid. Prepare In Small Quantities And Store In The Refrigerator. Shake Gently Before Use.

4.Christensen's Urease Test Medium

Peptone	Lg
Sodium Chloride	5g
Dipotassium Hydrogen Phosphate	2g
Phenol Red	6ml
Agar	20g
Distilled Water	1 Ltr
10% Sterile Solution Of Glucose	10ml
Sterile 20% Urea Solution	100ml

Sterilize The Glucose And Urea Solutions By Filtration. Prepare The Basal Medium Without Glucose And Urea, Adjust To Ph 6.8-6.9 And Sterilize By Autoclaving In A Flask At 121°C For 30min. Cool To About 50°C, Add The Glucose & Urea, And Tube The Medium As Slopes.

5.Simmon's Citrate Medium

Koser's Medium	1 Ltr
Agar	20 G
Bromothymol Blue 0.2%	40ml

Dispense, Autoclave At 121°C For 15 Min And Allow To Set As Slopes

6.Triple Sugar Iron Medium

Beef Extract	3g
Yeast Extract	3g
Peptone	20g
Glucose	Lg
Lactose	10 G
Sucrose	L0g
Ferric Citrate	0.3g
Sodium Chloride	5g
S OdumThiosulphate	0.3g
Agar	12g
Phenol Red 0.2% Solution	12ml
Distilled Water	1 Ltr

Heat To Dissolve The Solids, Add The Indicator Solution, Mix And Tube. Sterilize At 121°C For 15 Min And Cool To Form Slopes With Deep Butts.

7. Glucose Phosphate Broth

Peptone	5g
Dipotassium Hydrogen Phosphate	5g
Water	1 Ltr
Glucose 10% Solution	50ml

Dissolve The Peptone And Phosphate And Adjust The Ph To 7.6. Filter Dispense In 5ml Amounts And Sterilize At 121°C For 15min. Sterilize The Glucose Solution By Filtration And Add 0.25ml To Each Tube.

8.Peptone Water Fermentation Test Medium.

To The Basal Medium Of Peptone Water, Add Sterilised Sugars Of 1% Indicator Bromothymol Blue With Durham's Tube. Basal Medium Peptone Water Sugar Solutions:

Sugar	1ml
Dislilled Water	100ml

Ph = 7.6.

9.Potassium Nitrate Broth

Potassium Nitrate (Kno3)	0.2gm
Peptone	5.0gm
Distilled Water	100ml

The Above Ingredients Were Mixed And Transferred Into Tubes In 5 Ml Amount And Autoclaved.

10.Phenyl Alanine Deaminase Test

Yeast Extract	3g
Dl-Phenylalamine	2 g
Disodium Hydrogen Phosphate	L g
Sodium Chloride	5 g
Agar	12g
Distilled Water	1 Lr
Ph	7.4

Distributed In Tubes And Sterilized By Autoclaving At 121° C For 1 5 Minutes, Allowed To Solidify As Long Slopes.

11.POTASSIUM HYDROXIDE MOUNTS

It is prepared from the following ingredients

Potassium hydroxide : 20 gms

Glycerol : 10 ml

Distilled water : 80 ml

To a solution of 20% KOH, 10% Glycerol is added to prevent drying.Mix ingredients and store at room temperature. For 40% KOH 40gms of potassium hydroxide is added.

12.LACTOPHENOL COTTON BLUE STAIN

The lacto phenol cotton blue (LPCB) is used to study the morphological features of the fungal isolates.

It contains the following ingredients:

Melted phenol : 20 ml

Lactic acid : 20 ml

Glycerol : 40 ml

Cotton blue : 0.05 gm

Distilled water : 20 ml

Mix all the reagents properly and dissolve 0.05 g of cotton blue stain in the distilled water before mixing with the remaining reagents. The phenol acts as disinfectant, lactic acid preserves the morphology of the fungi and glycerol is hygroscopic agent which prevents drying. The cotton blue stains the outer wall of the fungus. Tease out of a fragment of the culture on a glass slide in a drop of LCB using two teasing needles. Put of a coverslip and examine under the microscope. If the plane LCB is used the edges of the coverslip can be sealed with nail polish to keep it for longer period of time.

13. Sugar Fermentation Medium

Peptone 15g	
Andrade's Indicator	10 Ml
Sugar To Be Tested	20g
Water	1 Litre

Andrade's Indicator Is Prepared From 0.5% Aqueous Acid Fuchsin to Which Sufficient 1m Sodium Hydroxide Has Been Added to turn the colour of theSolution Yellow.

Dissolve The Peptone And Andrade's Indicator In 1 Litre Of Water And Add 20g Of The Sugar; Sugars To Be Tested Generally Include Glucose, Sucrose, Lactose And Maltose. Distribute 3ml Amounts In Standard Test Tubes Containing An Inverted Durham Tube. Sterilize By Steaming At 100 Degree C For 30 Min On 3 Consecutive Days.

Annexure

ANNEXURE-1

PROFORMA

- Name : IP no:
- Age: Ward:
- Sex:
- Occupation:
- Address:

Presenting complaints:

- High grade fever-
- Chills:
- Lower abdominal pain
- Urinary incontinence
- Urinary urgency
- Urinary frequency

Past history:
H/O of trauma
Personal history:

- Alcohol intake:
- Cigarette smoking:

Associated immunocompromised state:

Physical examination:

UDS findings:

Cystoscopy findings:

CT scan findings/USG:

Provisional diagnosis:

Laboratory evaluation:

Biochemical parameters:

- plasma glucose levels

- Blood urea
- Creatinine

Microbiological investigation:
Sample collected:

- Urine

Direct examination:

Gram’stain:

KOH mount:

Bacterial Culture:

Sheep Blood agar

CLED agar

Fungal culture:

- SDA with antibiotics
- CHROMagar

Isolate identified in urinesample:

Antibacterial susceptibility pattern:

Antifungal susceptibility pattern:

ANNEXURE-2

PATIENT CONSENT FORM

STUDY TITLE:

A study on "Urinary bacterial and fungal isolates and their antimicrobial susceptibility pattern from patients undergoing invasive urological OP diagnostic procedures incuding Cystoscopy and Urodynamic study"

I……, hereby give consent to participate in the study conducted by Dr.M.Viji, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my urine sample for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal

Signature/ Thumb impression Place:

Of the patient/ relative

Date:

Patient Name & Address:

Signature of the investigator:

Signature of the guide:

ANNEXURE – IV - MASTER CHART CYSTOSCOPY

S.No	Name	Age	Sex	OP/IP No	Ward	Cystoscopy		Associated Conditions	Isolate				Antibiotic Sensitivity														
													Drugs														
						Indication	Findings		0	3	7	3 & 7	Ak	Gm	Nt	Nf	Cotri	Ctx	Cz	Cip	Amp	Pen	HLG	Ery	Cx	Flu	Vori	Pt
1	Rajeswari	60	F	10432	urology	Stent Removal	-	DM																				
2	Valliamma	60	F	2859	urology	LUTS for evaluation	N	DM																				
3	Veerayee	70	F	10082	urology	LUTS for evaluation	N	DM, HT		Klebsiella.spp			S	R	R	S	-	R	-	-	R	-	-	-	-	-	-	
4	Indrani	60	F	10931	urology	LUTS for evaluation	AN	Ca. cervix																				
5	Ravi	43	M	10581	urology	Stent Removal	-	DM																				
6	Bharathi	50	F	10796	urology	Stent Removal	-	DM		E.coli			S	S	S	S	-	S	-	-	R	-	-	-	-	-	-	
7	Chandrasekhar	63	M	9506	urology	Stent Removal	-	DM																				
8	Perumal	72	M	9744	urology	Stent Removal	-	DM		Acinetobacter.spp			S	S	S	S	-	S	-	-	R	-	-	-	-	-	-	
9	Lakshmanan	50	M	10583	urology	LUTS for evaluation	AN	DM																				
10	Parasuraman	60	M	10981	urology	Stent Removal	-	BPH		Proteus			S	R	R	S	-	R	-	-	R	-	-	-	-	-	-	
11	Rajeswari	56	F	10651	urology	Stent Removal	-	DM, HT																				
12	Ellamala	75	F	10828	urology	Stent Removal	-	DM, HT			E.coli		S	R	S	S	-	S	-	-	R	-	-	-	-	-	-	
13	Sudalai	52	M	5292	urology	Stent Removal	-	BPH																				
14	Puthira	60	F	10487	urology	Stent Removal	-	Ca. cervix		E.coli + Enterococci			S	S	S	S	-	S	-	-	R	-	-	-	-	-	-	
15	Rajiv Gandhi	26	M	9772	urology	Stent Removal	-	Meatal Stenosis																				
16	Lakshmi	62	F	10840	urology	Dysuria for evaluation	AN	HT, CVD																				
17	Ilanga	60	F	10652	urology	Stricture Urethra	N	DM, HT																				
18	Sivakumar	34	M	10735	urology	LUTS for evaluation	AN	HT, CVD		Klebsiella.spp			S	S	S	S	-	S	-	-	S	-	-	-	-	-	-	
19	Neelammal	55	F	10678	medicine	LUTS for evaluation	N	DM, HT																				
20	Dhanalakshmi	45	F	10901	urology	LUTS for evaluation	N	Leuchorea																				
21	Vennila	50	F	10567	urology	Dysuria for evaluation	AN	Prolapse																				
22	Jegatha	74	F	10902	urology	Stricture Urethra	AN	DM, HT																				
23	Ramu	27	M	10949	urology	Stent Removal	-	Meatal Stenosis		E.coli			S	R	S	S	-	S	-	-	S	-	-	-	-	-	-	
24	Mohana.P	23	F	10408	urology	LUTS for evaluation	AN	Leuchorea																				
25	Kanakaraj	68	M	9823	urology	LUTS for evaluation	N	DM			E.coli		S	R	S	S	-	S	-	-	R	-	-	-	-	-	-	
26	Susila	56	F	8379	urology	LUTS for evaluation	N	Ca. cervix		E.coli + CONS			-	-	S	S	S	-	-	-		R	-	S	S	-	-	
27	Lakshmi.K	50	F	10768	urology	Stent Removal	-	Ca. cervix																				
28	Deivayani	50	F	9142	urology	LUTS for evaluation	AN	Ca. cervix																				
29	Bakkiam	65	F	11080	urology	LUTS for evaluation	N	DM																				
30	Prakash	32	M	7659	urology	Stent Removal	-	DM																				
31	GunaPushpavanam	58	F	9468	urology	Uretric Calculii	AN	DM				E.coli	S	R	R	S	-	S	-	-	R	-	-	-	-	-	-	
32	Venkatraman	56	M	9052	urology	Uretric Calculii	AN	DM																				
33	Muniamma	60	F	10989	urology	Stent Removal	-	DM		E.coli			S	S	S	S	-	S	-	-	R	-	-	-	-	-	-	
34	Thunabai	55	F	10134	urology	Stent Removal	-	DM		E.coli			S	R	S	S	-	S	-	-	R	-	-	-	-	-	-	
35	Myikanni	50	F	17003	urology	Uretric Calculii	AN	Hernia																				
36	Munusamy	61	M	60846	urology	LUTS for evaluation	AN	HT, CVD																				
37	Rajeswari	52	F	11340	urology	LUTS for evaluation	AN	Ca. cervix		Proteus			S	S	S	S	-	S	-	-	R	-	-	-	-	-	-	
38	Kalairani	39	F	10456	urology	Stent Removal	-	Ca. cervix																				
39	Anvarul	27	M	10244	urology	Stent Removal	-	DM, HT																				

40	Chidambaram	65	M	11204	urology	Stent Removal	-	BPH		E.coli + Klebsiella.spp			S	R	S	R	-	S	-	-	R	-	-	-	-	-	-	
41	Sulochana	65	F	32124	urology	Renal Failure	AN	HT, CVD																				
42	Chandra	50	F	10757	urology	LUTS for evaluation	N	Leuchorea																				
43	Ponjolai	48	F	11296	urology	Stent Removal	-	Ca. cervix																				
44	Ambigai	45	F	11397	urology	Uretric Calculii	AN	DM																				
45	Manikkammal	61	F	11964	urology	B/L contracted Kidney	AN	HT, CVD			Proteus		S	S	S	S	-	S	-	-	S	-	-	-	-	-	-	
46	Lakshmi	35	F	108925	urology	LUTS for evaluation	AN	Leuchorea																				
47	Bannibai	54	F	11491	urology	Stent Removal	-	DM																				
48	DuraiRaj	53	M	11866	urology	Stent Removal	-	BPH			E.coli		S	R	S	S	-	S	-	-	R	-	-	-	-	-	-	
49	Poongavanam	58	F	11849	urology	LUTS for evaluation	N	DM, HT		E.coli			S	R	S	S	-	R	-	-	R	-	-	-	-	-	-	
50	Diwakar	19	M	8115	urology	Stent Removal	-	Meatal Stenosis																				
51	Ponnappan	40	M	10851	urology	LUTS for evaluation	AN	DM																				
52	Chinnapillai	65	M	41654	urology	LUTS for evaluation	N	HT, CVD			Acinetobacter.spp		S	R	-	S	-	-	S	S	-	-	-	-	-	-	-	S
53	Karpakammal	70	F	6140	urology	LUTS for evaluation	N	Ca. cervix																				
54	Parameshwari	58	F	10405	urology	Renal Failure	AN	HT, CVD																				
55	Veerammal	48	F	11987	urology	Uretric Calculii	AN	DM		Klebsiella.spp			S	S	S	S	-	S	-	-	R	-	-	-	-	-	-	
56	Elumalai	60	M	44193	urology	LUTS for evaluation	AN	BPH																				
57	Indira	58	F	32426	urology	LUTS for evaluation	AN	DM			E.coli + Candida spp		S	R	S	S	-	S	-	-	R	-	-	-	-	-	-	
58	Samboornamma	68	F	32455	urology	LUTS for evaluation	N	Ca. cervix		Candida.spp			-	-	-	-	-	-	-	-	-	-	-	-	-	S	S	
59	Baskar	42	M	32540	urology	LUTS for evaluation	N	DM, HT																				
60	NandaGopal	55	M	32556	urology	Stent Removal	-	DM, HT			Klebsiella.spp		S	R	S	S	-	S	-	-	R	-	-	-	-	-	-	
61	Ravikumar	50	M	40684	urology	Stent Removal	-	BPH																				
62	Kaliappan	42	M	44084	urology	Stent Removal	-	DM																				
63	Selvaraj	39	M	43928	urology	LUTS for evaluation	N	HT, CVD																				
64	Srinivasan	46	M	205354	urology	Stent Removal	-	DM																				
65	Chinnaiyya	75	M	42782	urology	Stent Removal	-	DM		Pseudomonas aeruginosa			S	R	-	S	-	-	S	R	-	-	-	-	-	-	-	S
66	Krishnan	45	M	24385	urology	Stent Removal	-	DM																				
67	Govindaraj	46	M	44365	urology	Stent Removal	-	Meatal Stenosis																				
68	Barith	60	M	44198	urology	Stent Removal	-	BPH				E.coli	S	S	R	S	-	S	-	-	R	-	-	-	-	-	-	
69	Murthi	60	M	42291	urology	LUTS for evaluation	AN	DM																				
70	Mariyaraj	78	M	5164	urology	LUTS for evaluation	N	BPH																				
71	Arumugam	70	M	2356	urology	LUTS for evaluation	AN	DM, HT		Klebsiella.spp			R	R	S	S	-	S	-	-	R	-	-	-	-	-	-	
72	Kishore	55	M	4435	urology	LUTS for evaluation	N	DM		Candida albicans			-	-	-	-	-	-	-	-	-	-	-	-	-	S	S	
73	Bala	50	F	44030	urology	B/L contracted Kidney	N	HT, CVD																				
74	Shanmugam	55	M	7561	urology	Stent Removal	-	BPH																				
75	Selvakumar	42	M	7549	urology	Dysuria for evaluation	N	Renal artery stenosis		E.coli + Enterococci			S	S	S	R	-	S	-	-	R	-	-	-	-	-	-	
76	Vellalan	80	M	2883	urology	Stricture Urethra	N	BPH																				

77	Muruga Pandiyan	43	M	89849	urology	Stricture Urethra	AN	DM		E.coli			S	S	S	S	-	S	-	-	R	-	-	-	-	-	-	
78	Muthu	51	M	9840	urology	Dysuria for evaluation	AN	DM, HT																				
79	Abdul Munat	75	M	7510	urology	LUTS for evaluation	AN	DM		Acinetobacter.spp			S	R	S	S	-	S	-	-	R	-	-	-	-	-	-	
80	Rajendran	53	M	41468	urology	LUTS for evaluation	AN	BPH																				
81	Sudhakar	53	M	4475	urology	LUTS for evaluation	N	BPH																				
82	Jothi	38	F	6151	urology	Stricture Urethra	N	DM, HT																				
83	Gopinath	34	M	4477	urology	Stricture Urethra	AN	Acute pyelo nephritis																				
84	Chandran	75	M	44691	urology	Renal Failure	AN	BPH			Enterococci		-	-	S	S	-	-	-	S	R	R	S	-	-	-	-	
85	Narayanan	52	M	4461	urology	Stent Removal	-	BPH		Proteus			S	S	S	S	-	S	-	-	R	-	-	-	-	-	-	
86	Balakumar	62	M	2443	urology	Dysuria for evaluation	AN	BPH																				
87	Sivakumar	36	M	32899	urology	LUTS for evaluation	N	HT, CVD																				
88	Rajangam	46	M	32461	urology	Dysuria for evaluation	AN	COPD		Staph.aureus			-	-	S	S	S	-	-	-	-	R	-	S	R	-	-	
89	Ponnusamy	45	M	32854	urology	Uretric Calculii	AN	DM		Proteus			S	S	S	S	-	S	-	-	R	-	-	-	-	-	-	
90	Manohar	59	M	44325	urology	Stent Removal	-	DM, HT																				
91	Syed Gaffer	80	M	44378	urology	B/L contracted Kidney	AN	DM, HT, CVD		Pseudomonas aeruginosa			S	R	-	S	-	-	S	R	-	-	-	-	-	-	-	S
92	Ibaih	40	M	41067	urology	LUTS for evaluation	AN	HT, CVD																				
93	Viswanathan	33	M	33046	urology	LUTS for evaluation	N	DM																				
94	Kempu	59	M	32529	urology	LUTS for evaluation	AN	DM		E.coli			S	R	S	S	-	S	-	-	R	-	-	-	-	-	-	
95	Munusamy	50	M	32545	urology	Stent Removal	-	DM		E.coli			S	S	S	S	-	R	-	-	R	-	-	-	-	-	-	
96	Venkalamma	52	F	3274	urology	Dysuria for evaluation	N	Leuchorea			Enterococci		-	-	S	S	-	-	-	S	R	R	S	-	-	-	-	
97	Changamma	67	F	44128	urology	Stricture Urethra	AN	DM, HT		Proteus			S	R	R	R	-	S	-	-	R	-	-	-	-	-	-	
98	Kaveri	60	F	2411/14	urology	Stent Removal	-	DM, HT																				
99	Nandhiyamma	55	F	32643	urology	Uretric Calculii	AN	DM			Candida spp		-	-	-	-	-	-	-	-	-	-	-	-	-	S	S	
100	Neelarani	55	F	4325	urology	Stent Removal	-	DM																				

MASTER CHART - URODYNAMIC STUDY

S.No	Name	Age	Sex	OP/IP No	Ward	Urodynamic Study	Associated Conditions	Isolate				Antibiotic Sensitivity														
												Drugs														
								0	3	7	3 & 7	Ak	Gm	Nt	Nf	Cotri	Ctx	Cz	Cip	Amp	Pen	HLG	Ery	Cx	Flu	Vori
1	Meenachi	55	F	4460	Uro-OP	Mixed Incontinence	DM																			
2	Uma	38	F	40712	Uro-OP	Urge incontinence	COPD		E.coli			S	S	S	S	-	S	-	-	R	-	-	-	-	-	-
3	Mumtaj	42	F	4462	Uro-OP	Urge incontinence	Recurrent UTI																			
4	Vimala	60	F	7579/64	Uro-OP	Mixed Incontinence	DM																			
5	Malar	45	F	4473	Uro-OP	Mixed Incontinence	Recurrent UTI																			
6	Devaki	40	F	4475	Uro-OP	Mixed Incontinence	Recurrent UTI																			
7	Selvi	37	F	4476	Uro-OP	Mixed Incontinence	COPD																			
8	Ambika	35	F	90631	Uro-OP	Mixed Incontinence	PUO																			
9	LeenaMary	35	F	4482	Uro-OP	Urge incontinence	Recurrent UTI		Klebsiella.spp			S	R	S	S	-	S	-	-	R	-	-	-	-	-	-
10	Jaya	45	F	42712	Uro-OP	Renal artery stenosis	COPD																			
11	Munusamy	75	M	44409	Uro-OP	Neurogenic bladder	Hemiplegia																			
12	Dhanammal	58	F	36272	Uro-OP	Urge incontinence	DM																			
13	Latha	27	F	4810	Uro-OP	Urge incontinence	Trauma																			
14	Usha	49	F	4870	Uro-OP	Mixed Incontinence	Recurrent UTI																			
15	Lakshmi	43	F	94393	Uro-OP	Mixed Incontinence	PUO		Klebsiella.spp + CONS			S	R	S	S	-	S	-	-	R	-	-	-	-	-	-
16	Pallavi	70	F	44965	Uro-OP	Mixed Incontinence	Hemiplegia																			
17	Kalaiyarasan	36	M	33065	Uro-OP	Mixed Incontinence	HT/CVA			E.coli		S	R	S	S	-	R	-	-	R	-	-	-	-	-	-
18	Ganesan	49	M	33031	Uro-OP	Mixed Incontinence	HT/CVA																			
19	Sakubai	60	F	33034	Uro-OP	Mixed Incontinence	DM																			
20	Saminath	55	M	32958	Uro-OP	Mixed Incontinence	DM																			
21	Ramalingam	49	M	32941	Uro-OP	Mixed Incontinence	HT/CVA																			
22	Srinivasan	64	M	33038	Uro-OP	Mixed Incontinence	DM/CVA																			
23	Abdul Mohammed	75	M	32905	Uro-OP	Mixed Incontinence	Hemiplegia		E.coli			S	R	R	R	-	S	-	-	R	-	-	-	-	-	-
24	Kaliyammal	60	F	32895	Uro-OP	Mixed Incontinence	DM																			
25	Marudhammal	65	F	32873	Uro-OP	Mixed Incontinence	DM																			
26	Poongavanam	75	M	7263	Uro-OP	Mixed Incontinence	DM/CVA																			
27	Subramani	67	M	287891	Uro-OP	Urge incontinence	DM																			
28	Subban	70	M	32023	Uro-OP	Neurogenic bladder	DM																			
29	Parthasarathy	49	M	30029	Uro-OP	Urge incontinence	HT/CVA																			
30	Jeeva	35	M	7404	Uro-OP	Mixed Incontinence	Trauma																			
31	Shanmugam	68	M	4389	Uro-OP	Mixed Incontinence	DM		Klebsiella.spp			S	R	S	S		R	-		R						
32	Srinivasan	48	M	8615	Uro-OP	Mixed Incontinence	Recurrent UTI																			
33	Samraj	45	M	43366	Uro-OP	Mixed Incontinence	COPD																			
34	Kalimuthu	65	M	42903	Uro-OP	Mixed Incontinence	DM																			
35	Seemapandiyan	46	M	38724	Uro-OP	Mixed Incontinence	HT																			
36	Ramesh	35	M	36434	Uro-OP	Mixed Incontinence	HT/CVA																			
37	Raja	34	M	42063	Uro-OP	Mixed Incontinence	HT/CVA																			
38	Jongan	59	M	40587	Uro-OP	Mixed Incontinence	NeurogenicBladder				E.coli	S	R	S	S	-	S	-	-	R	-	-	-	-	-	-
39	Muniammal	60	F	43087	Uro-OP	Mixed Incontinence	HT/CVA			Candida spp + E.coli		-	-	-	-	-	-	-	-	-	-	-	-	-	S	S
40	Babu	47	M	42626	Uro-OP	Mixed Incontinence	PUO																			

41	Venkatesan	42	M	32088	Uro-OP	Mixed Incontinence	HT/CVA																			
42	Thanikachalam	68	M	42187	Uro-OP	Mixed Incontinence	HT/CVA																			
43	Thangavel	76	M	32107	Uro-OP	Neurogenic bladder	HT/CVA																			
44	Manohar	50	M	32119	Uro-OP	Urge incontinence	HT/CVA																			
45	Logaraj	57	M	32131	Uro-OP	BPH	DM		E.coli			S	R	S	S	-	S	-	-	R	-	-	-	-	-	-
46	Kaliyamurthi	57	M	32134	Uro-OP	Urge incontinence	DM																			
47	Chinnaiyan	72	M	32136	Uro-OP	Mixed Incontinence	Hemiplegia																			
48	Mani	44	M	32149	Uro-OP	Urge incontinence	Recurrent UTI																			
49	Rajan	64	M	32150	Uro-OP	Mixed Incontinence	DM																			
50	Baskar	50	M	32257	Uro-OP	Urge incontinence	DM																			
51	Lakshmanan	56	M	32169	Uro-OP	Urge incontinence	DM		Enterococci			-	-	S	S	-		-	R	R	R	S	-	-	-	-
52	Ramadoss	53	M	60649	Uro-OP	Mixed Incontinence	DM																			
53	Mariappan	56	M	4346	Uro-OP	BPH	DM																			
54	Pandiyan	43	M	7499	Uro-OP	Urge incontinence	Recurrent UTI																			
55	Saravanan	40	M	7500	Uro-OP	Renal artery stenosis	COPD																			
56	Munusamy	40	M	32523	Uro-OP	Urge incontinence	PUO		E.coli			S	S	S	S	-	S	-	-	R	-	-	-	-	-	-
57	Veeramani	72	M	32057	Uro-OP	Mixed Incontinence	DM																			
58	Balakumar	62	M	32519	Uro-OP	Urge incontinence	DM/CVA																			
59	Ramadoss	30	M	32478	Uro-OP	Mixed Incontinence	Recurrent UTI																			
60	Muna	70	F	32046	Uro-OP	Neurogenic bladder	Hemiplegia																			
61	Sundari	52	F	7396	Uro-OP	Mixed Incontinence	DM																			
62	Ezhila	41	F	87214	Uro-OP	Urge incontinence	Dysuria																			
63	Raj	45	M	4116	Uro-OP	Urge incontinence	DM																			
64	Laxmi	66	F	31353	Uro-OP	Mixed Incontinence	DM																			
65	Amirtha	70	F	37443	Uro-OP	Mixed Incontinence	DM																			
66	Jayapriya	42	F	43323	Uro-OP	Urge incontinence	PUO																			
67	Kokila	55	F	41634	Uro-OP	Mixed Incontinence	DM		Proteus			S	R	S	S	-	S	-	-	R	-	-	-	-	-	-
68	Kasthuri	58	F	39217	Uro-OP	BPH	DM																			
69	Dhanalakshmi	43	F	41463	Uro-OP	Urge incontinence	COPD																			
70	Vijaya	45	F	43488	Uro-OP	Mixed Incontinence	DM																			
71	Pushpa	52	F	32170	Uro-OP	Mixed Incontinence	DM																			
72	Jayalakshmi	85	F	32275	Uro-OP	Mixed Incontinence	DM																			
73	Venkata Subbama	60	F	32198	Uro-OP	Urge incontinence	DM																			
74	Ananthana	70	F	32184	Uro-OP	Neurogenic bladder	Hemiplegia																			
75	Raju	57	M	45031	Uro-OP	BPH	DM																			
76	Yasodha	60	F	7459	Uro-OP	Urge incontinence	DM																			
77	Meena	35	F	7498	Uro-OP	Mixed Incontinence	Trauma																			
78	Thamayanthi	75	F	30393	Uro-OP	Urge incontinence	Hemiplegia																			
79	Sivapoorana	70	F	32389	Uro-OP	Neurogenic bladder	Hemiplegia				Klebsiella.spp	S	R	S	S	-	S	-	-	R	-	-	-	-	-	-
80	Sakunthala	75	F	323441	Uro-OP	Neurogenic bladder	DM																			
81	Amsammal	60	F	32329	Uro-OP	Mixed Incontinence	DM																			
82	Harabhagyam	47	F	32342	Uro-OP	Urge incontinence	PUO																			
83	Amaravathy	55	F	7501	Uro-OP	Mixed Incontinence	DM																			
84	Vagappan	54	M	58422	Uro-OP	Mixed Incontinence	hemiparesis																			
85	Chakkaravarthy	62	M	64721	Uro-OP	Neurogenic bladder	DM/CVA																			
86	Kumar	36	M	61631	Uro-OP	Urge incontinence	PUO																			
87	Murugeasan	63	M	60339	Uro-OP	Mixed Incontinence	DM																			

88	Venkateshwaran	47	M	60277	Uro-OP	BPH	HT		E.coli + Proteus			S	R	R	S		S	-		R						
89	Pappathiyamma	70	F	8491	Uro-OP	Neurogenic bladder	DM/HT																				
90	Samayanthan	68	M	726538	Uro-OP	Mixed Incontinence	DM/CVA																				
91	Ramamoorthy	64	M	82182	Uro-OP	Urge incontinence	HT																				
92	Chandra	60	F	82184	Uro-OP	Mixed Incontinence	Leucorrhea			Proteus		S	R	S	S	-	S	-	-	R	-	-	-	-	-	-
93	Valli	48	F	82526	Uro-OP	Urge incontinence	DM																				
94	Balakrishnan	47	M	76046	Uro-OP	Stress incontinene	HT/CVA																				
95	Vennkatesan	49	M	82088	Uro-OP	Detrusor instability	DM/HT																				
96	Kathavarayan	85	M	69308	Uro-OP	BPH	DM																				
97	Mani	45	M	73092	Uro-OP	Stress incontinene	Trauma																				
98	Sundari	40	F	8444	Uro-OP	Urge incontinence	Dysuria																				
99	Janakirani	50	F	8455	Uro-OP	Mixed Incontinence	Leucorrhea																				
100	Elumalai	26	M	1442	Uro-OP	Stress incontinene	Trauma																				

KEY TO MASTER CHART

Ak	-	Amikacin
Gm	-	Gentamycin
Nf	-	Nitrofurantoin
Nx	-	Norfloxacin
Ctx	-	Cefotaxime
Amp	-	Ampicillin
Cz	-	Ceftazidime
Pt	-	PiperacillinTazobactum
Hlg	-	High level gentamycin
Flu	-	Flucanazole
Vor	-	Voricanazole
Ery	-	Erythromycin
CX	-	Cefoxitin
Cotri	-	Cotrimoxazole
Pen	-	Penicillin
Cip	-	Ciprofloxacin
S	-	Sensitive
R	-	Resistant

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