*IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*
*e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 22, Issue 7 Ser.3 (July. 2023), PP 26-43*
*www.iosrjournals.org*

# Title

AUTHOR

Date of Submission: 26-06-2023 Date of Acceptance: 06-07-2023

## I. INTRODUCTION

Among the most common infectious diseases, urinary tract infections (UTIs) are commonly encountered diseases by clinicians in developing countries with an estimated annual global incidence of at least 250 million. UTIs refer to the presence of microbial pathogens within the urinary tract and it is usually classified by the infection site:-bladder [cystitis], kidney [pyelonephritis], or urine [bacteriuria] and also can be asymptomatic or symptomatic, UTIs that occur in a normal genitourinary tract with no prior instrumentation are considered as "uncomplicated," where as "complicated" infections are diagnosed in genitourinary tracts that have structural or functional abnormalities, including instrumentation such as in dwelling urethral catheters, and are frequently asymptomatic. It has been estimated that globally symptomatic UTIs result in as many as 7 million visits to outpatient clinics, one million visits to emergency departments, and 100,000 hospitalizations annually. According to WHO the incidence rate of Urinary tract infection (UTI) is 0.91% of total population and 2.05% for a female population with *Escherichia coli* being the most common pathogen accounting for 80% to 90% of all cases. Many different microorganisms can cause UTIs though the most common pathogens causing the simple ones in the community are *Escherichiacoli* and other Enterobacteriacae, which accounts approximately 75% of the isolates. In complicated urinary tract infections and hospitalized patients, organisms such as *Enterococcus faecalis* and highly resistant Gram-negative rods including *Pseudomonas* spp. are comparatively more common**(1)**.The relative frequency of the pathogens varies depending upon age, sex, catheterization, and hospitalization.

Treatment of UTI cases is often starts empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens **(2)**. However, a large proportion of uncontrolled antibiotic usage has contributed to the emergence of resistant bacterial infections. As a result, the prevalence of antimicrobial resistance among urinary pathogens has been increasing worldwide. Associated resistance, i.e. the fact that a bacterium resistant to one antibiotic is often much more likely to be resistant to other antibiotics, drastically decreases our chances of getting a second empirical attempt right. Resistance rates to the most common prescribed drugs used in the treatment of UTIs vary considerably indifferent areas world-wide. The estimation of local

etiology and susceptibility profile could support the most effective empirical treatment. Therefore, investigating epidemiology of UTIs(prevalence, risk factors, bacterial isolates and antibiotic sensitivity) is fundamental for caregivers and health planners to guide the expect editor venations**(3).**Biofilm impact humans in many ways as they can from in natural, medical and industrial settings. For instance, formation of biofilm on medical devices, such as catheters or implants often results in difficult-to-treat chronic infections. Moreover, infection have been associated with biofilm formation on human surfaces such as teeth, skin and the urinary tract**(4).**Microorganism attach to surface and develop biofilm-associated cell can be differentiated from their suspended counterparts by generation of an extracellular polymeric substance (EPS) matrix**(5).**

**AIMS AND OBJECTIVES**

- To study the lactose and non -lactose fermenting bacteria and their antibiotic resistance causing urinary infection.
- To study the biofilm producing uropathogen by tube method and congo red agar method.

## II. REVIEW OF LITERTURE

Urinary Tract Infection (UTI) is caused by both Gram-negative and Gram-positive, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogen *Escherichia coli*(UPEC) followed by *Klebsiellapneumoniae*, *Staphylococcus saprophyticus, Enterococcus faecalis, Group B streptococcus*, *Proteus mirabilis, Pseudomonas aeuginosa*, *Staphylococcus aureus*, and *Candida spp*. For complicated UTIs, the orders of prevalence for causative agents are; UPEC as most common followed by*Entrococcus spp. K. pneumoniae, Candida spp., S. aureus, P.mirabilis, P. aeruginosa*.

Urinary tract infection (UTI) is defined as the presence of microbial pathogen in the urinary tract with associated symptoms. When it affects the lower urinary tract it is known as cystitis and when it affects the upper

urinary tract it is known as pylonephritis. The infection process may involve the kidney, renal pelvis, ureters, bladder and urethra along with adjacent structures, such as prostate and epididymis in males. Urinary tract infections are important complications of diabetes, renal disease, renal transplantation and structural neurological abnormalities that interfere with urine flow. In addition urinary tract infections are the leading cause of Gram negative sepsis in hospitalized patients and are the origin for about half of all nosocomial infections caused by urinary catheters **(3).**

The incidence of urinary infection is greatly influenced by age, sex and by predisposing factors that impair the defence mechanism that maintain the sterility of the normal urinary tract. Females are more prone to suffer from urinary tract infection because of short urethra and are in close proximity of anus and urethral trauma during intercourse. 20-50% of women have urinary tract infection at some time in their life and a significant number have recurrent infections. Although majority of infections are acute and short lived, they contribute to a significant amount of morbidity and health care expenditure in the population.

The prevalence of bacteriuria in females increases gradually with time to as high as 10% to 20% in elderly women. Result of anatomic and hormonal changes during pregnancy can cause urinary tract infection and can lead to serious complications in both mother and foetus. Studies have shown that incidence of bacteriuria (presence of bacteria in urine) among girls aged 5 to 14 is 1-2%. Infections in children are often hard to recognize because of their variable symptomology and the difficulty of obtaining suitable urine samples.

In males urinary tract infections are uncommon, except in first year of life. In male patients over 60 years it is specially related to enlargement of prostate or instrumentation interfering with emptying of bladder.

Normal urine is mostly water, salt (sodium and chloride ions) and urea. The yellow color comes from the pigment urochrome which is left over from bilirubin after red blood cells have been recycled and is sterile, but it is free of bacteria, viruses, and fungi. An infection occurs when microorganisms, usually bacteria from the digestive tract, cling to the opening of the urethra, begin to multiply and travel up to the bladder known as ascending route. For urinary tract infections to occur by the ascending pathway, enteric gram negative bacteria and other microorganism that originate in the gastro intestinal tract must be able to colonize the periurethral area. Once these organisms gain access to the bladder, they may multiply and then pass up the ureters and kidney. Enteric bacteria (in particular, Escherichia coli) have been and remain the most frequent cause of UTI, although there is some evidence that the percentage of UTIs caused by E. coli is decreasing **(6).**

There are two Pathwaysin the pathogenesis of recurrent UTI, frequent repeat ascending infection and chronic infection in the bladder. Each pathway also might result from two possible mechanisms, bacterial factor and deficiencies in host defense.

Adherence is a key event initiating each step in UTI pathogenesis. A UTI typically starts with periurethral contamination by a uropathogen residing in the gut, followed by colonization of the urethra and subsequent migration of the pathogen to the bladder, an event that requires appendages such as flagella and pili (FIGURE. 1). In the bladder, the consequences of complex host–pathogen interactions ultimately determine whether uropathogens are successful in colonization or eliminated.Multiple bacterial adhesins recognize receptors on the bladder epithelium (also known as the uroepithelium) and mediate colonization. Uropathogens such as UPEC survive by invading the bladder epithelium, producing toxins and proteases to release nutrients from the host cells, and synthesizing siderophores to obtain iron. By multiplying and overcoming host immune surveillance, the uropathogens can subsequently ascend to the kidneys, again attaching via adhesins or pili to colonize the renal epithelium and then producing tissue-damaging toxins. Consequently, the uropathogens are able to cross the tubular epithelial barrier to access the blood stream, initiating bacteraemia.

The uropathogens that cause uncomplicated UTIs, including UPEC, *K. pneumoniae* and *S. saprophyticus*, have the ability to bind directly to the bladder epithelium, which is composed of the umbrella cells (also known as superficial facet cells), intermediate cells and basal cells. UPEC and *K. pneumoniae* bind to uroplakins (urothelium specific trnsmembrane proteins), which are the major protein components of the umbrella cell apical membrane and which form a crystalline array protecting the mammalian bladder tissue from damaging agents in urine. In addition to uroplakins, α3β1 integrins, which are expressed at the surface of uroepithelial cells, can also serve as receptors for UPEC. By contrast, complicated UTIs are initiated when the bacteria bind to a urinary catheter, a kidney stone or a bladder stone, or when they are retained in the urinary tract by a physical obstruction. Some pathogens (for example, UPEC) can cause both uncomplicated and complicated UTIs. However, others such as *P. mirabilis*, *P. aeruginosa* and *Enterococcus* spp. predominantly cause complicated UTIs**(7).**

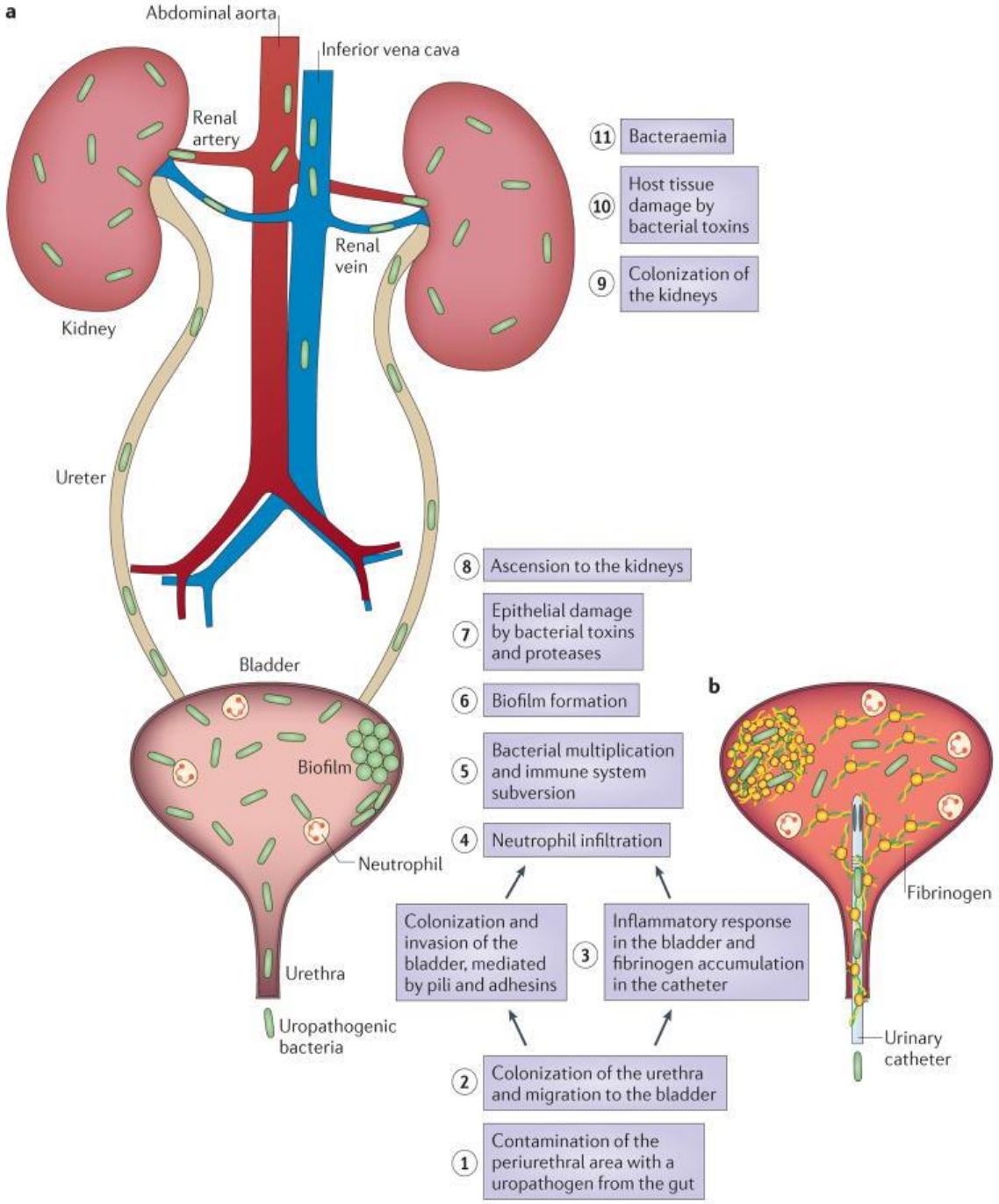



**Figure: Pathogenesis of urinary tract infections**

**Deficiencies in host defense**

It is well known that patient withimmunodeficiency tend to have frequent, recurrent and severe UTIs. However, recurrent UTI in someimmunocompetentpatientmight also be caused by host defense deficiencies **(7).**

Virulence factors of uropathogenic E. coli that have been potentially implicated as important to establish UTIs can be divided into two group:

1. Virulence factor associated with the surface of bacteria cell and
2. Virulence factorswhich are secreted and exported to the site of action.

**Surface Virulence factors,** Uropathogenic*Escherichia coli* include a number of different types of adhesive fimbriae, which promote bacterial attachment to host tissue within the urinary tract.

The capsule provides protection against phagocytic engulfment and complement-mediated bactericidal effect in the host. Certain capsule types, for example,K1 and K5, show a molecular mimicry to tissue components, preventing a proper humoral immune response of the infected host.

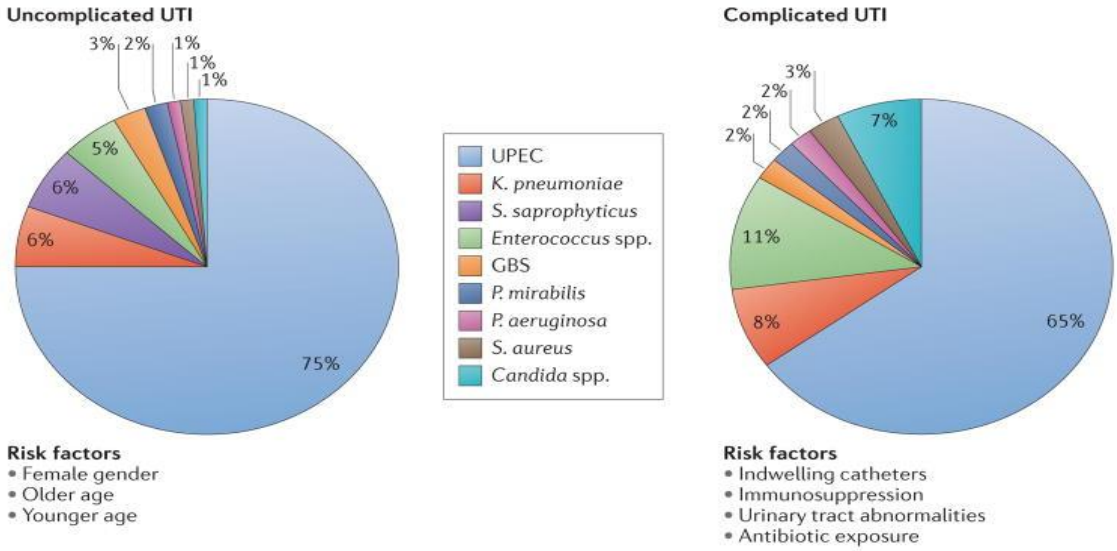
TheLPS of uropathogenic*Escherichia coli* integral component of the cell well of Gram-negative bacteria.LPS is known to activate host response and to induce nitric oxide and cytokine production. Although LPS of UPEC is important in activation of proinflammatorresponse in uncomplicated UTIs,it is not clear whether LPS plays a role in mediating a renal failure and acute allograft injury in patients with ascending UTIs. Acute failure due to LPS depends on the systemic response to LPS and does not depend on expression of functional LPS receptor TLR4, in the kidney. However, TLR4 is expression in renal epithelia and in the renal pelvis, and these findings suggest that the ascending infection due to *Escherichia coli*may stimulate the innate immune response associated with the acute allograft injury in patients with UTIs.

Flagella,an organelle responsible for bacterial motility, are involved in the interaction of various pathogenic *Escherichia coli* stains with epithelial cells. Flagellated uropathogenic*Escherichia coli* cause 70 to 90% of all urinary tract infections **(8).**

**SecretedVirulence Factors,**toxins are important virulence factors in a variety of *Escherichia coli*-mediate diseases. Production of toxin by colonizing *Escherichiacoli* may cause an inflammation response, a possible pathway for UTIs symptoms. The most important secreted virulence factors of uropathogenic*Escherichia coli* is a lipoprotein called alpha-haemolysin (HlyA), which is associated with upper UTIs such as pyelonephritis. This toxin has been shown to exert dual concentration-dependent activities on primary epithelial cells originating from renal proximal tubules. At high concentration, HlyA is able to lyses erythrocytes and nucleated host cells. At low concentration, HlyA can induce the apoptosis of target host cells, and promote the exfoliation of bladder epithelial cells. Alpha-haemolysin has been shown to induce calcium oscillation in renal epithelial cells, resulting in increased production of IL-6 and IL-8. Approximately 50% of all cases of pyelonephritiswhich leads prevents bacterial adherence by forming a mucin layer.The low pH and presence of salts, urea, and organic acids in urine can reduce bacterial survival within the urinary tract.

**Epidemiology:**

Urinary tract infections (UTIs) are caused by a wide range of pathogens, including Gram-negative and Gram-positive bacteria, as well as fungi. Uncomplicated UTIs typically affect women, children and elderly patients who are otherwise healthy. Complicated UTIs are usually associated with indwelling catheters, urinary tract abnormalities, immunosuppression or exposure to antibiotics. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC). For uncomplicated UTIs, other causative agents are (in order of prevalence) *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *GroupB Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida spp*. For complicated UTIs, the other causative agents are *Enterococcus spp, K. pneumoniae, Candida spp, S. aureus, P. mirabilis, P aeruginosa***(7).**




**Specimen collection,**

Suprapubic aspiration is the best method to avoid contamination of specimen with bacteria in the distal urethra. The collection method is used infrequently because it is invasive and uncomfortable, and it requires too much time and too many resources to be practical. Collection of urine by use of a single catheter is the next-best technique for obtaining urine specimen with minimal contamination. Most urine samples arecollected by the clean-catch midstream technique.This technique has the following advantages: it is neither invasive nor

uncomfortable, it is simple and inexpensive, it can be performed in almost any clinical setting, there is no risk of introducing bacteria into the bladder by catheterization, and there is no risk of complications. Colony counts from urine specimens collected by this method correlate reasonably well with those of specimens collected via suprapubic aspiration or straight catheterization **(9, 10).**

**Isolationofbacteria**

The urinary tract is a typically sterile environment, which is maintained by a variety of host mechanisms to preventbacterial colonization and survival. Most of the pathogenic bacteria, which cause UTIs, are from the host own bowel flora and enter the bladder via the urethra. Uroepithelial adherence is critical for establishment of UTIs. Clinical symptoms may sometimes be a good initial guide to the presence and site of infection, but many infections are symptomless, and genital infection may mimic infections of the urinary tract. Urine culture may form 25-40% of the work in average clinical laboratory. Cultural techniques are employed not only to detect bacteria but also enumerate bacteria in the urine. The organisms are identified by semiquantitative or quantitative cultures and their susceptibility to antimicrobial agents is determined. Resistant organisms are most often associated with infection acquired in hospitals and are cause of complicated urinary tract infections.

Several studies have demonstrated the adverse effect of delays in transportation or processing of urine specimens on their quality. On the basis of the results of these and other similar studies, it is currently recommended that urine specimens be plated within 2 h after collection unless specimens have been refrigerated or kept in a preservative**(11).**

Specimen processing: Routine urine cultures should be plated using calibrated loops for the semiquantitative method. This method has the advantage of providing information regarding the number of cfu/mL (colony forming units) as well as providing isolated colonies for identification and susceptibility testing. The types of media used for routine cultures should be limited to blood agar and MacConkey's agar orcystinine lactose electrolyte deficient (CLED) Agar **(10).**

Pyuria can be detected and quantified microscopically by measuring the urinary leukocyte excretion rate, counting leukocytes in uncentrifuged specimen. Leukocytes should be found in number of at least greater as $10^4$/ml before pyuria is established (12)**.**

**Sensitivity to antibiotics**

Form the use of first line drugs of antibiotics are: determining sensitive and resistant bacteria to antibiotics by measuring the diameter of inhibition zone by mm and then compared with the standard diameters that installed in the standard scales **(9).**

**Biofilm**

Biofilms have been described in many systems since Van Leeuwenhoek examined the “animalcules” in the plaque on hisown teeth in the seventeenth century, but the general theory of biofilm predominance was not promulgated until 1978. Costerton et al. observed that communities of attached bacteria in aquatic systems were found to be encased in a “glycocalyx” matrix that was found to be polysaccharide in nature, and this matrix material was shown to mediate adhesion**(13).**

The term ‘film’, which refers to bacterial adhesion, aggregation, and multiplication on surfaces, was used in marine microbiology to distinguish adhering (sessile) bacteria from free-swimming ‘planktonic’ organisms from 1933 to 1935. Henrici used direct microscopy to study biofouling in fresh water and he observed that “it is quite evident that for the most part water bacteria are not free floating organisms, but grow attached upon submerged surfaces **(14,15,16).**

Biofilm are defined as microbial derived sessile communities characterized by the cells that are irreversibly attached to a substratum or to each other. They are embedded in a matrix of extracellular polymeric substances (EPS) they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription. Within a biofilm, bacteria communicate with each other by production of chemotactic particles or pheromones, a phenomenon called quorum sensing. Availability of key nutrients, chemo taxis toward surface, motility of bacteria, surface adhesions and presence of surfactants are some factor which influence biofilm formation **(17).**

**Formation of Biofilm**

Biofilms may form on a wide variety of surface, including living tissues, indwelling medical devices, industrial or potable water system piping, or natural aquatic systems**(5).**

**Steps for Biofilm formation:**

**I. Reversible attachment of planktonic bacteria to surface.** The first attachment of the bacteria is influenced by attractive or repelling forces that vary depending on nutrients levels, pH, and the temperature of the site or niche. In this step, flagella and chemotaxis play an important role avoiding the action of the hydrodynamic and repulsive forces as well as selecting the surface**(18).**

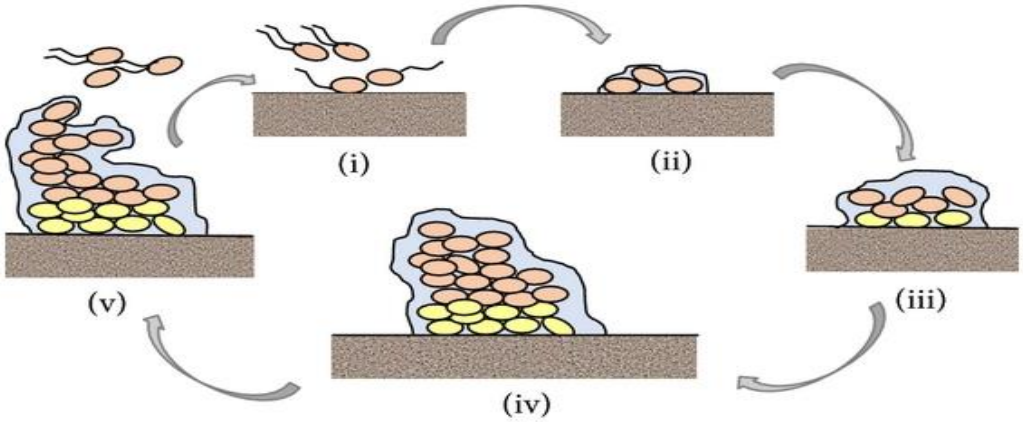



**FIGURE 2: Biofilm formation steps. (1) Reversible attachment of planktonic bacteria to surface. (2) Irreversible attachment to surface. (3) Formation of the external matrix. (4) Biofilm acquire a three dimensional structure. (5) Biofilm detachment.**

**II.** ***Irreversible attachment to surface.***In the case of *Escherichia coli*, it is mediated by type 1 pili, curli fibers, and antigen 43 that also favours the interbacterial interaction **(19).**

**Formation of a complex layer of biomoleculers and EPS secretion that constitute the external matrix.**Production of polysaccharides in biofilm forming stains facilitates aggregation, adherence, and surface tolerance, allowing better surface colonization. The *Escherichia coli* matrix is composed of cellulose, polyglucosamine, and colonic acid. The *P. aeruginosa* matrix is composed two types of polysaccharides: the capsule and aggregative polysaccharides. Alginate is the main and most studied capsular polysaccharides produced by *Pseudomonas aeruginosa* and maintains the characteristics of protective dynamic polymers that present one or more cells. On the other hand, aggregative polysaccharides confer structural integrity to biofilm. Nucleic acids, such as DNA, protein, surfactants, lipids, glycolipids, membrane, vesicles, and ions such as calcium can also be found forming part of the matrix composition and may play an important role in the characteristics that biofilm structure confers to the cells**(18).**

**Biofilm acquire a three-dimensional structure when they reach maturity.** Theyreach maturity. These three-dimensional structures with macrocolony morphology depend on self-produced extracellular matrix components. EPS, adhesins,amyloid-forming protein, and exopolysaccharides (all included in biofilm matrix) are required to generate these structures in which gradients of nutrients, water, signaling compounds or waste products are present along the different areas of biofilm, conditioning the metabolism of the cells.

**III. When biofilm are fully mature, detachment may occur.** Detachment allows cells to again take on a planktonic state and can thereby form biofilm in setting. It has been proposed that bacteria detachment could be caused by active mechanisms initiated of the bacteria themselves such as enzymatic degradation of the biofilm matrix and quorum sensing in response to environmental changes related to nutrition levels and oxygen depletion and by passive mechanisms mediated by external forces and erosion biofilm dispersal is an important step in a high transmission from environment to human host, between host, and even within a signal host spreading the infection**(18).**

**Mechanism of biofilm**

The first step of biofilm generation requires microorganisms to adhere to a surface. The adhesion is initially weak, as it is achieved through van der Waals forces; an important aspect is the reversible character of biofilm generation at this initial stage. In other word, should any factors act to detach the microbes from the respective surface during this early stage, biofilm formation would be prevented. Whenever there is no external early action taken against microbial at attachment to that surface, the adherence will increase and tend to become permanent due to the involvement of cell adhesion structures e.g, pili. Thus, in the case of an implanted

medical device or equipment which has been contaminated by microorganisms, the chance of further biofilm development with consecutive infection depends on number of variables. As stated above, the presence of microorganism on the device must last long enough in order for the initial stage of weak, reversible attachment to be transformed into the quasi-permanent adherence. The number and type of cell in the exposure environment of the implanted device, the liquid flow rate (in the case of human devices), and the physic-chemical structure of the implanted material may be listed among the most important variables involved.

**Bacteria growth and multiplication supported by biofilm**

Biofilm formation is a naturally occurring phenomenon encountered in the external environment and impacting various aspects of human life, such as contamination of foods, corrosion and/or obstruction of pipes, etc. Similarly, such phenomena also occur within the human body resulting, for instance, in dental plaque formation, mastitis, otitis, pneumonia, urinary tract infection **(13).**

**Dispersal of biofilm**

Biofilm bacteria can move in numerous ways that allow them to easily infect new tissue. Biofilm may move collectively, by rippling or rolling across the surfaces, or by detaching in clumps. Sometime, in a dispersal strategy referred to as "Swarming/seeding", a biofilm colony differentiates to form an outer "wall" of stationary bacteria, white the inner region of the biofilm "liquefies" allowing planktonic cell to " Swim" of the biofilm and leave behind a hollow mound **(20).**

**Cell Death and Cell lysis**

Several studies have shown that biofilm dispersal is preceded or accompanied by lysis of a subpopulation of cell within macrocolonies of mature biofilm **(21, 22).**

**FACTORS AFFECTING BIOFILM FORMATION**

I. **Attachment**

The solid-liquid interface between a surface and an aqueous medium (eg water, blood) provides an ideal environment for the attachment and growth of microorganisms **(5).**

II. **Substratum Effects**

Charackilis et al. noted that the extent of microbial colonization appear to increase as the surface roughness increases. This is because shear forces are diminished, and surface area is higher on rougher surface. The physicochemical properties of the surface may also exert a strong influence on the rate and extent of attachment **(23).**

**BIOFILM AND DEVICES ASSOCIATED INFECTIONS**

Bacteria can attach to and infected all medical devices and up to 60% of HAIs are associated with biofilm infection of implantable medical devices. The biofilm on medical devices are composed of gram-positive and gram-negative bacteria, or yeast. Most commonly isolated bacteria include gram-positive organism such as *Enterococcus fecalis, Staphylococcus aureus, Staphylococcus Epidermidis*, *Streptococcus viridians* and gram-negative organisms like *Escherichia coli*, *Klebsiella pneumonia, Proteus mirabilis and Pseudomonas aeruginosa.* Indwelling devices can be colonized with single or multiple species bacteria. In the case of urinary catheters, initially the biofilm are composed of a single species and continued further exposures lead to multispecies biofilm**(24).**

These are several factors that influence the rate and extent of biofilm formation on devices. First the bacteria must attach to the surface of the devices long enough to result in permanent attachment. The initial rate of attachment depends on the number and type of bacteria cells in the fluid in which the devices is exposed to, the flow rate through the devices and the physicochemical characteristics of the exposed surface**(5)**.

**PATHOGENESIS OF DEVICE RELATED INFECTIONS**

Device-related infection results from the multifaceted interaction of bacteria, device, and host factors. Of these 3 factors, bacterial factor is probably the most important in the pathogenesis of device-associated infection, whereas device factors are the most amenable to modification with objective of preventing infection **(25).**

**Bacterial factors**

Different bacteria use different adhesion to colonize medical devices. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the two most commonly associated and studied organisms in relation to device related infections.

- *Staphylococcus aureus* appears to be more dependent on the presence of host-tissue ligands, including fibronectin, fibrinogen, and collagen.
- Persistent adherence of gram-negative bacteria is thought to be associated with presence of fimbriae.

**i. Device Related Factors**

The presence of the device can, in and of itself, enhance bacterial virulence. Careful analysis of the data on bacteria adherence and surface modification of the device yields the following 5 major principles **(25).**

(1).Different bacteria may adhere differently to the same device material

(2).The same bacteria may adhere differently to different device materials.

(3).The same bacteria may adhere differently to the same device material placed under different circumastances, including the medium in which the device is placed (hydrophobic vs. hydrophilic medium), type of flow does not (dynamic vs. stationary), and temperature.

(4).In vitro inhibition of bacteria colonization of the device does not ensure anti-infective efficacy in vivo.

(5).The clinical benefit of a particular surface-modifying approach may vary from one application to another.

**Table :Device related factors that may favour bacterial adherence.**

**Device-related factors that may favor bacterial adherence**

| | |
|---|---|
| **Type of Device material** | • Polyvinyl chloride favors bacterial adherence more than does Teflon<br>• Polyethylene favors bacterial adherence more than does polyurethane<br>• Latex favors bacterial adherence more than dose silicone<br>• Silicone favors bacterial adherence more than does polytetrafluoroethylene<br>• Stainless steel favors bacteria adherence more than does titanium |
| **Source of Device material** | • Synthetic favors bacterial adherence more than does biomaterial |
| **Surface of Device** | • Irregular favors bacterial adherence more than does regular<br>• Textured favors bacterial adherence more than does smooth<br>• Hydrophobic favors bacterial adherence more than does hydrophilic |
| **Shape of Device** | • Polymeric tubing favors bacterial adherence more than does wire mesh |

**Host Related Factors**

Immune-mediated phenomena that promote bacterial persistence are illustrated by the reduced complement opsonic activity and the decreased bactericidal activity of WBCs in tissue surrounding the implanted device. The most studied immune mediator that can inhibit persistence of already adherent bacteria on the surface of the device is IFN-gamma **(26).**

**MIDROBIAL ADHERENCE TO DEVICE**

Immediately upon insertion of a medical device into the patient, macromolecules such as fibrinogen and immunoglobulins are deposited on the implants surface. This is called the conditioning film and generally makes it easier for bacteria to attach to the implant. Several proteinproduced by staphylococcus produce protein that bind specifically to host factors in the conditioning film. Bacteria can randomly come in contact with implant surface by sedimentation and Brownian motion while motion microbial cells may actively seek the implant surface. Initial bacteria adhesion occurs due to van der Waals forces and surface structure such as fibrils or polymers create a link between the substrate and individual bacteria. Once attached, the bacteria produce diffusible signaling molecules in a process called quorum sensing which, among other things, induces the bacteria to secrete EPS. It is the EPS that cement the bacteria to each other and surface and provides mechanical stability for the three-dimensional development of the biofilm. The biofilm structure itself is dynamic with redistribution of reversibly-attached cells, recruitment of cells from the surrounding fluid, replication of attached cells and dispersal of cells back into the surrounding areas**(27).**

**Table: Biofilm-associated microorganisms commonly isolated from selected indwelling medical device**

| **Indwelling medical device** | **Organisms** |
|---|---|
| Central venous catheter | Coagulase-negative *Staphylococci, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans* |
| Prosthetic heart valve | *Viridians streptococcus coagulase-negative Staphylococci, Enterococci, Staphylococcus aureus* |
| Urinary catheter | *Escherichia coli, Staphylococcus epidermidis, klebsiella pneumonia, Enterococcus faecalis, Proteus mirabilis* |
| Artificial hip prosthesis | *Coagulase-negative Staphylococci, Beta-hemolytic Streptococci, Enterococci, Proteus mirabilis, Bacteriodesspp, Staphylococcus aureus, Viridians Streptococcus, Escherichia coli, Pseudomonas aeruginosa* |
| Artificial voice prosthesis | Staphylococcus *epidermis Candida albicans, Streptococcus mitis, Streptococcus salivarius, Rothia*Staphylococcus*dentrocanosa, Candida tropicalis,* |
| Intrauterine devices | *epidermidis, Corynebacterium spp, Staphylococcus aureus, Micrococcus spp, Lactobacillus plantanum, group B Streptococcus, Enterococcus spp, Candida albicans* |

## III. MATERIALS AND METHODS

**Study type**

Prospectivecross-sectional study.

**Specimen collection:**

All urine samples received in Central Lab Microbiology section were included in this study. Samples were collected after proper instruction to the patient. Detailed history was recorded in case recording form.

**Sample size and processing:**

A total of 400 urine samples were included in the study. All samples were inoculated in CLED agar (Cysteine lactose electrolyte deficient agar) with Bromothymol blue indicator and were incubated under aerobic condition at 37° for 24 hours. Microscopy of uncentrifused urine was also done to see pus cells **(28).**50µl(1 drop) sample was taken on a clean glass slide and observed by putting a cover slip under low power microscope. Lactose and non-lactose fermenting bacteria was identified by colony characteristics on CLED media. Morphology of the organism was determined by gram stain.

**Automated identification and Antimicrobial susceptibility**

The identification and antimicrobial sensitivity was done by Vitak automated system with ID and AST (280 and 281) card.

This system (VITEK 2 Compact) is used for the identification and antibiotic susceptibility profile of the bacterial and yeast isolates.

VITEK 2 Compact is an automated microbiology system utilizing growth-based technology. It constitutes colorimetric reagent cards that are incubated and interpreted automatically. This system also provides an option of automatic pipetting and dilution for antimicrobial susceptibility testing.

The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances. Each card has a pre-inserted transfer be used for inoculation. Cards have bar codes that contain information on product type, lot number, expiration date and a unique identifier that can be linked to the sample either before or after loading the card onto the system.

GN- Gram-negative fermenting and non-fermenting bacilli

GP- Gram-positive cocci and non-spore-forming bacilli

**Biofilm detection**

**1Tube method:**

a) A loopful of test organism was inoculated in 10ml of trypticase soya broth with 1% glucose in test tube

The tube was incubated at 37°c or 24 hours. After incubation tube was decanted and washed with phosphate buffer saline (Ph 7.3) dried tubes was then stained with crystal violet (0.1%) excess stain was washed with deionized water. Tubes were dried in inverted position**(17).**

**Congo red Agar Method**

Composition:

| | |
|---|---|
| Brain heart infusion broth | 37g/L |
| Sucrose | 50g/L |
| Agar | 10g/L |
| Congo red indicator | 0.8g/L |

- First Congo red stain was prepared as a concentrated aqueous solution and autoclaved (121° C for 15 minutes ) separately from the other medium constituents
- Then it was added to the autoclaved brain heart infusion agar with sucrose at 55°C.
- Congo red agar plates was inoculated with test organisms and incubated at 37°C for 24 hours aerobically.
- Black colonies with a dry crystalline consistency indicated biofilm production.

## IV. RESULT

A total of 400 urine samples were received in the Central laboratory, SGRRIM & HS, Patel Nagar Dehradun. Out of which 102 were positive for aerobic growth and 298 were sterile. Few of which were mixed growth was repeated with proper collection.

**Table 1: Distribution of samples**

| | |
|---|---|
| Positive culture | 102 |
| Sterile | 298 |
| **Total** | 400 |

The above table showing 102 positive culture among total urine sample (400) collected.

**Table 2: Gender-wise distribution of positive cases (n=102)**

| Gender | Number of cases | Percentage (%) |
|---|---|---|
| Male | 46 | 45.09% |
| Female | 56 | 54.90% |
| **Total** | **102** | **100%** |

As per the above table female preponderance was more 55% against male 45%.

**Figure 1: Gender-wise distribution of Culture Positive Cases (n=102)**

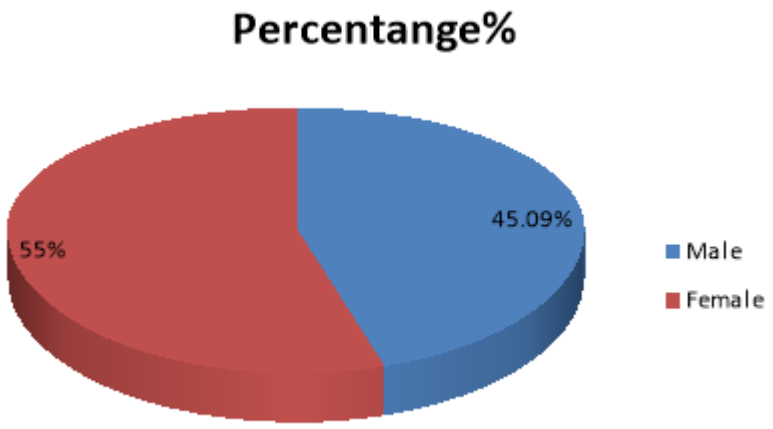



**Table 3: Age-wise distribution of sample Cases (n=102)**

| Age Group (in years) | Numbers of Cases | Percentage of Cases |
|---|---|---|
| 1-10 | 5 | 4.90% |
| 11-20 | 3 | 2.94% |
| 21-30 | 24 | 23.52% |
| 31-40 | 14 | 13.72% |
| 41-50 | 12 | 11.76% |
| 51-60 | 14 | 13.72% |
| 61-70 | 21 | 20.58% |
| 71-80 | 9 | 8.82% |

21-30 (23.52%) years age group showed positivity followed by 61-70 (20.58%) years of age.

**Figure 2: Age-wise distribution of positive cases (n=102)**

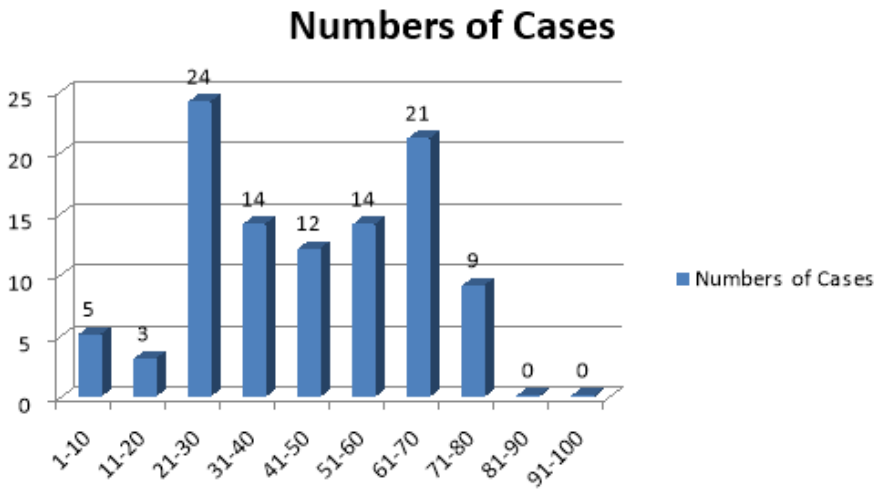



**Table 4: Spectrum of organisms isolated from patients (n=102)**

| Organism isolated | Number of cases | Percentage of cases |
|---|---|---|
| *Escherichia coli* | 66 | 67.32% |
| *Klebsiella pneumoniae* | 12 | 12.24% |
| *Pseudomonas aeruginosa* | 8 | 8.16% |
| *Enterococcus faecalis* | 5 | 5.10% |
| *Enterococcus faecium* | 3 | 3.06% |
| *Proteus mirabilis* | 3 | 3.06% |
| *Enterobacter aerogenes* | 2 | 2.04% |
| *Enterococcus gallinarum* | 1 | 1.02% |
| *Klebsiella oxytoca* | 1 | 1.02% |
| *Providencia rettgeri* | 1 | 1.02% |
| **Total** | **102** | **100%** |

Maximum number of organism isolated as *Escherichia coli* (56.68%) followed by *Klebsiella pneumonae* (10.78%), *Pseudomonas aeruginosa* (8.16%), *Enterococcus faecalis* (5.10%), *Enterococcus faecium* (3.06%), *Proteus mirabilis* (3.06%), *Enterobacter aerogenes* (2.04%), *Enterococcus gallinarum* (1.02%), *Klebsiella oxytoca* (1.02%), *Providencia rettgeri* (1.02%).

**Figure 3: Spectrum of organisms isolated from patients (n=102)**

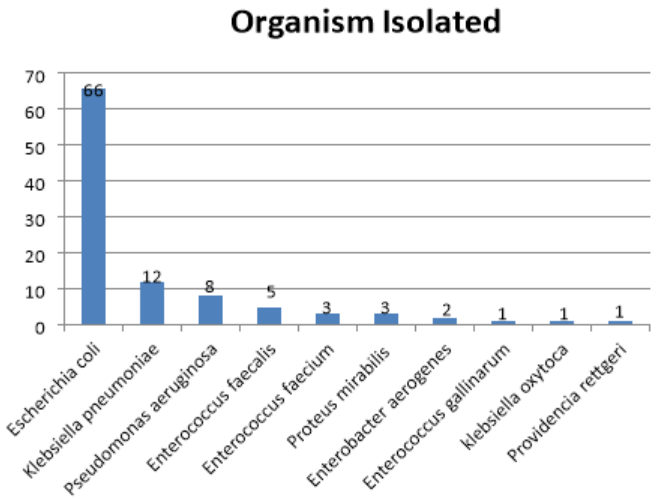


**Table 5: Distribution of gram positive and gram negative bacteria among positive cases**

| Organisms | **Number of organisms** | **Percentage(%)** |
|---|---|---|
| Gram positive | **09** | **9** |
| Gram negative | **93** | **91** |
| Total | **102** | **100** |

The above table shows 91% gram negative isolates and 9% gram positive.

**Table 6: Distribution of lactose and Non-lactose fermenting bacteria (n=93)**

| **Organisms** | **Number of organisms** | **Percentage (%)** |
|---|---|---|
| **Lactose fermenting** | 81 | 87 |
| **Non lactose fermenting** | 12 | 13 |
| **Total** | 93 | 100 |

Out of 93 Gram negative 81(87%) were lactose fermenter and 12 (12%) were NLF.

**Table 7:Ward-wise distribution of culture positive (n=102)**

| **Ward** | Number of cases | Percentage (%) |
|---|---|---|
| **SICU** | 2 | 1.96% |
| **MICU** | 5 | 4.90% |
| **HDU** | 2 | 1.96% |
| **Medicine** | 13 | 12.74% |
| **Surgery** | 17 | 16.66% |
| **Obstetrics** | 17 | 16.66% |
| **Gynecology** | 13 | 12.74% |
| **Orthopedics** | 1 | 0.98% |
| **Nephrology** | 10 | 9.80% |
| **Neurology** | 8 | 7.84% |
| **Urology** | 7 | 6.86% |
| **Peadiatrics** | 7 | 6.86% |
| **Total** | 102 | 100% |

The above table it is evident that maximum number of cases were from OBS & Gyne (29%) Surgery (16.6%) and Medicine (12.7%)

**Figure 4: Ward-wise distribution of culture positive cases (n=102)**

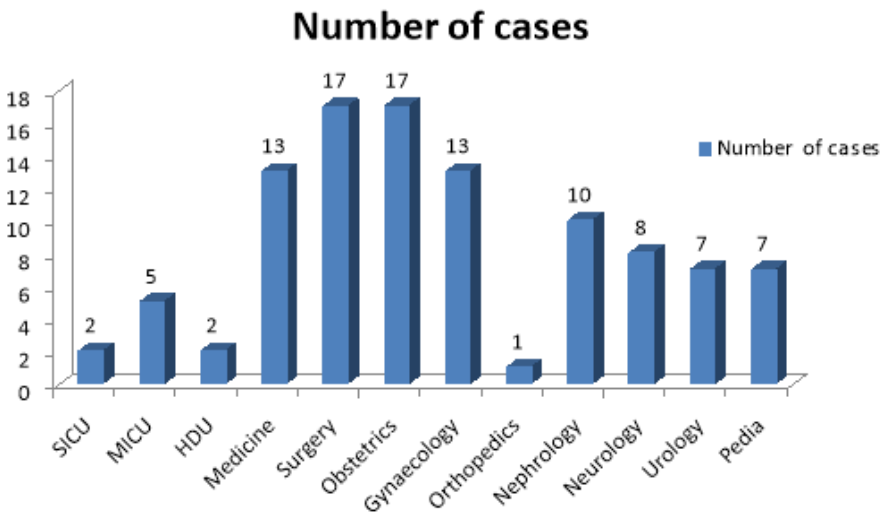


**Table 8: Patients showing associated symptoms among positive cases (n=102)**

| Complaints | Number of cases | Percentage |
|---|---|---|
| **Fever** | 16 | 15.68% |
| **Lower abdominal pain** | 21 | 20.58% |
| **Dysuria** | 3 | 2.94% |
| **Asymptomatic** | 62 | 60.78% |

As per the above table 60.78% patients were asymptomatic.

**Figure 5: Patients showing associated symptoms among positive cases (n=102)**

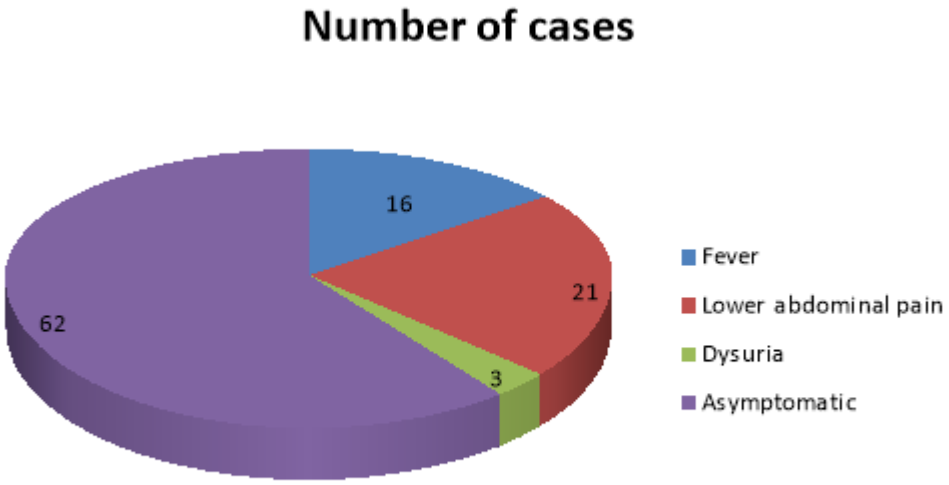



**Table 9: Organisms wise evaluation of different methods for biofilm detection (n=102)**

| Method | Weak/Non biofilm producers (%) | Moderate (%) | Strong (%) |
|---|---|---|---|
| **Congo red agar** | 40 (40.8%) | 25 (25.5%) | 37 (37.74%) |
| **Tube method** | 27(27.54%) | 65 (66.3%) | 10 (10.2%) |

As per above table by congo red method 25 were moderate and 37 strong biofilm producer. Whereas by tube method 65 moderate and 10 strong biofilm producer.

**Table 10: Percentage of Gram Positive bacteria and Gram Negative bacteria showing Biofilm production (n=102)**

| Method | Weak/Non biofilm producers | Moderate (%) | Strong (%) |
|---|---|---|---|
| **GRAMNEGATIVE** | | | |
| **Congo red agar** | 35 | 21 | 37 |
| **Tube method** | 24 | 59 | 10 |
| **GRAMPOSITIVE** | | | |
| **Congo red agar** | 5 | 4 | 0 |
| **Tube method** | 3 | 6 | 0 |

As per table above weak biofilm producer were more in congo red agar (35 in GNB/5 in GPC) and by tube method moderate biofilm producer are more (59 in GNB/ 6 in GPC) in both gram negative and gram positive. Strong biofilm producer in congo red agar was 37 and 10 were in tube method.

**Figure 6: Gram Positive and Gram Negative bacteria showing biofilm production (n=102)**

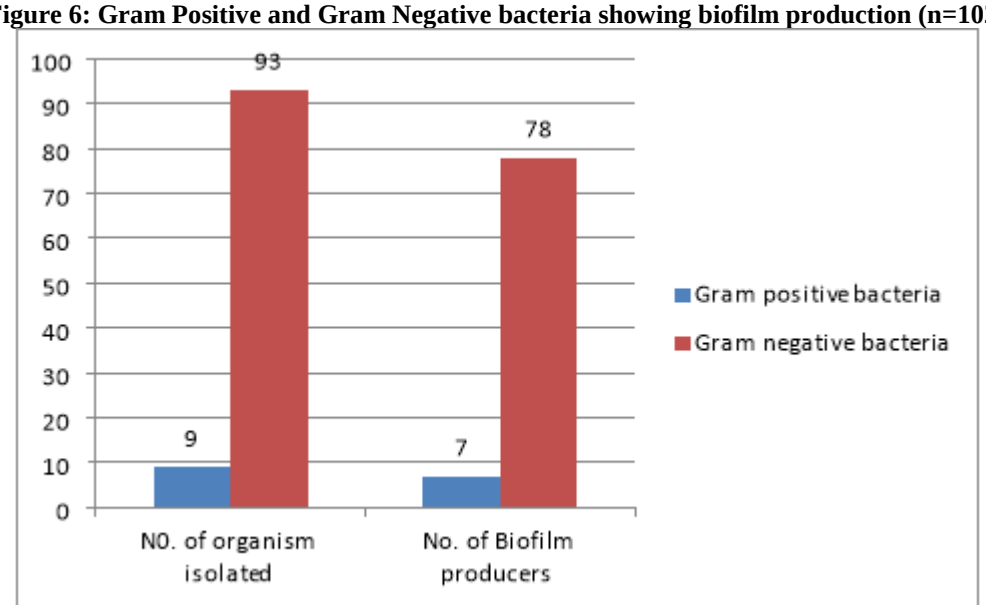
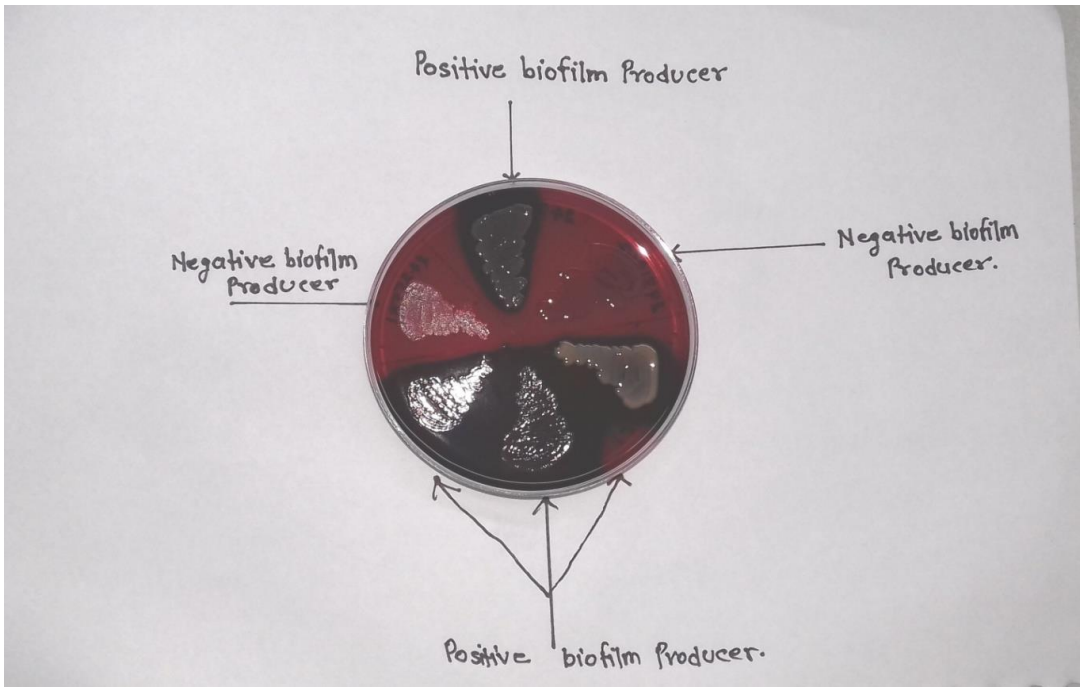


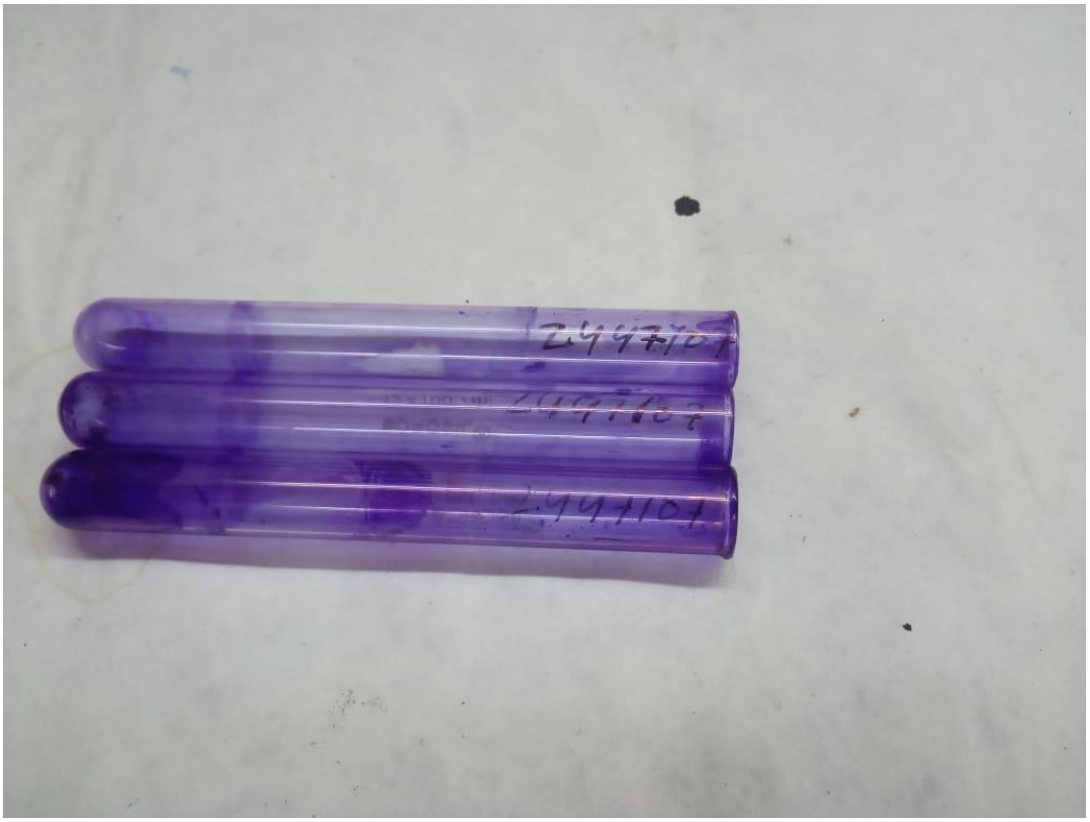

**Table 10: Resistance pattern of isolates**

| **Antibiotics** | **Escherichia coli (n=66)** | **Klebsilla spp (n=31)** | **Pseudomonas (n=8)** |
|---|---|---|---|
| Ampicillin | 86.15% | 91.66% | - |
| Amoxicillin/Clavulanic acid | 36.92% | 41.66% | - |
| Ampicillin/Sulbactam | 60.93% | 54.54% | - |
| Piperacillin/Tazobactam | 15.15% | 46.15% | - |
| Cefazolin | 67.69% | 72.72% | 100% |
| Ceftazidime | 46.96% | 58.33% | 66.66% |
| Ceftriaxone | 63.07% | 66.66% | - |
| Cefepime | 33.33% | 46.15% | 71.42% |
| Imipenem | 15.15% | 41.15% | 71.42% |
| Gentamicin | 31.81% | 38.46% | 33.33% |
| Tobramycin | 33.84% | 45.45% | 100% |
| Ciprofloxacin | 69.69% | 46.15% | 85.71% |
| Levofloxacin | 75.00% | 33.33% | 85.71% |
| Nitrofurantoin | 9.37% | 45.45% | - |
| Trimethoprim/Sulfamethoxazole | 74.24% | 46.15% | - |
| Ertapenem | 5.17% | 15.38% | - |
| Aztreonam | | 7.69% | 50% |
| Doripenem | 1.51% | 7.69% | 50% |
| Meropenem | 1.51% | 7.69% | 50% |
| Cefoperazone/Sulbactam | - | 7.69% | 75% |
| Tigecycline | - | - | 100% |
| Colistin | - | - | 25% |
| Ticarcillin/Clavulanic acid | - | 7.69% | 75% |
| Amikacin | - | - | 50% |
| | | | |
| | | | |

As per the above table maximum resistance was seen with Ampicillin for *E.coli* (86.15%) and minimum resistance with meropenem. For *Pseudomonas* maximum resistance (100%) was seen with Cefazolin and minimum resistance Colistin (25%)




**Photograph:1: Picture showing growth on Congo red agar.**



## V. DISCUSSION

The aim of the present study was to determine the incidence of lactose and non lactose fermenting bacteria and their antibiotic resistance causing significant bacterial urinary tract infection by using various culture techniques and bacteriological examination of clean catch midstream urine. and to see biofilm producing uropathogen by tube method and congo red agar method.

Out of total of 400 urine samples suspected cases of urinary tract infection, culture positive cases were 102 and sterile were 298 ( **Table: 1**). Lactose and non-lactose fermenting bacteria was identified by colony characteristics on CLED media. Morphology of the organism was determined by gram stain.

Microbiologists need to interpret the microbiologic relevance of growth on culture plates to determine whether further identification and antimicrobial susceptibility testing are necessary. Most culture results can be interpreted readily; no growth and gross contamination are both unambiguous results, as are pure cultures of common pathogens growing in a quantity of >105 cfu per milliliter of urine. The interpretation of cultures that yield pure growth in lower quantities is also clear for specimens obtained via suprapubic aspiration or straight catheterization **(12).** (**Akmal Hasan SK, 2014**).

The incidence of urinary infection is greatly influenced by age, sex and by predisposing factors that impair the defence mechanism that maintain the sterility of the normal urinary tract. Females are more prone to suffer from urinary tract infection because of short urethra and is in close proximity of anus and urethral trauma during intercourse (12). (**Akmal Hasan SK, 2014**). In this present study 46 (45%) cases were from male and 56 (55%) cases were from female (**Table: 2; Figure:1**). The age-wise distribution of cases shows maximum number of positive cases in age-group of 21-30 (23.52%) years and 61-70 years (20.58%) and minimum no of cases were in age-group of 11-20 years (2.94%) (**Table:3**). A study by Bharti Singh et al., showed increased risk of infection in 15-30 age group than 31-45 age group in females females. Women of 15-30 years age group were reported to have two folds increase UTI as compared to the 31-45 age groups (9). ( **Bharti Singh et al 2018**). Adult women are 30 times more likely than men to develop a UTI, with almost half of them experiencing at least one episode of UTI during their life time. UTIs are most commonly seen in sexually active young women. Other susceptible adults include the elderly and patients requiring urethral catheterization (29). (**Chee Wei Tan,MMed,MCFP,Maciej Piotr Chlebicki Urinary tract infections in adults. Singapore Med J. 2016;57(9):485-490.doi:10.11622/smedj.2016153).**

The urinary tract is a typically sterile environment, which is maintained by a variety of host mechanisms to prevent bacterial colonization and survival. Most of the pathogenic bacteria, which cause UTIs, are from the host own bowel flora and enter the bladder via the urethra. Uroepithelial adherence is critical for establishment of UTIs (8). (**Justyna Bien; 2012**). Among the positive cases; the organisms (**Table: 4**) are *Escherichia coli* (56.68%) followed by *Klebsiella pneumonae* (10.78%), *Pseudomonas aeruginosa* (8.16%), *Enterococcus faecalis* (5.10%), *Enterococcus faecium* (3.06%), *Proteus mirabilis* (3.06%), *Enterobacter aerogenes* (2.04%), *Enterococcus gallinarum* (1.02%), *Klebsiella oxytoca* (1.02%), *Providencia rettgeri* (1.02%).

**Table:5** shows organism isolated as gram positive are 9 (9%) and 93 (91%) gram negative organism. Among gram negative 81 were lactose fermenter **(Table: 6)** and 12 non lactose fermenter.

The treatment strategy for complicated UTIs depends on the severity of the illness and hospitalization is often necessary. *E.coli* is the predominant uropathogen isolated in acute, community acquired uncomplicated UITs in adults and children. Recommended antibiotics for acute uncomplicated cystitis are Fosfomycin, nitrofurantion (for women); amoxicillin- clavulanate (for men), Ciprofloxacin, cephalosporin(cephalexin, cefurixime), co-trimoxazole (if resistance is known for *E.coli*). Some of these antibiotics such as fosfomycin and nitrofurantoin are promptly excreted in urine and very low levels are found in tissues making them excellent choices for pyelonephritis **(29)**.

(**Chee Wei Tan,MMed,MCFP,Maciej Piotr Chlebicki Urinary tract infections in adults. Singapore Med J. 2016; 57(9):485-490.doi:10.11622/smedj.2016153).**

Principles for treating recurrent complicated UTIs include early use of broad-spectrum antibiotics, with adjustment of antibiotic coverage based on culture results and attempts to relieve any existing urinary obstruction based on results of imaging studies. Recommended oral antibiotic options include fluoroquinolones, amoxicillin- clavulanate and aminoglycosides (29).

The our study **Table: 6** showing maximum number of cases obtained from Surgery and Obstetrics (16.66% each) where as only one case was obtained from the Department of Orthopedics (0.98%)

**Table 9** showed biofilm production of organisms by congo red and tube method. By congo red method 25 were moderate and 37 (37.74%) strong biofilm producer. Whereas by tube method 65 moderate and 10 (10.2%) strong biofilm producer.

A study by Afreenish Hassan et al., (15) detected 49% isolates as biofilm producers and 51% as non-biofilm producers by tube method. By this method, three isolates were found to be false positive and 19 were false negative. TM is 73% sensitive, 92.5% specific and 80% accurate for biofilm detection. This method correlated well with tissue culture plate for identifying strong biofilm producers, but it was hard to differentiate between moderate, weak and non-biofilm producers due to the changeability in the results detected by different observers (17).

(Afreenish Hassan et al.Dis vol.15 no.4 Salvador July/Aug.2011).

In another study, Ruzicka et al.,(30) noted that out of 147 isolates of *S. epidermidis*, TM detected biofilm formation in 79 (53.7%) and CRA detected in 64 (43.5%) isolates. They showed that tube method is better for biofilm detection than CRA (31).

(Ruzicka F, Hola V, Votava M et al. Biofilm detection and clinical significance of *Staphylococcus epidermidis* isolates. Folia Microbiol (Praha) 2004; 49(5): 596-600.)

Baqai et al., tested TM to detect biofilm formation among uropathogens. According to their results, 75% of the isolates exhibited biofilm formation (32).

( **Baqai R, Aziz M, Rasool G. Urinary tract infection in diabetic patients and biofilm formation of uropathogens. Infect Dis J Pakistan. 2008 17(1):7-9.**

## VI. CONCLUSION

1. UTIs are a significant clinical problem. Uncomplicated UTIs are usually treated empirically with antibiotics.
2. All urine samples received in Central Lab Microbiology section were included in this study for identification AST and detection of biofilm in lactose and non lactose fermenting bacteria.
3. A total of 400 urine samples were received out of which 102 were positive for aerobic growth and 298 were sterile.
4. The identification and antimicrobial sensitivity was done by Vitak automated system with ID and AST(280 and 281) card.
5. Maximum number of organism isolated as *Escherichia coli* (56.68%) followed by *Klebsiella pneumonae* (10.78%), *Pseudomonas aeruginosa* (8.16%).
6. Out of 93 Gram negative bacilli 81(87%) were lactose fermenter and 12 (12%) were NLF.
7. In our study maximum number of cases were from OBS & Gyne (29%) Surgery (16.6%) and Medicine (12.7%).
8. 60.78% patients were having asymptomatic UTI.
9. Congo red method showed 25 moderate and 37 strong biofilm producer. Whereas by tube method 65 moderate and 10 strong biofilm producer were.
10. In this study maximum resistance was seen with Ampicillin for *E.coli* (86.15%) and minimum resistance with meropenem. For *Pseudomonas* maximum resistance (100%) was seen with Cefazolin and minimum resistance Colistin (25%). Resistance of Nitrofurantoin is 9.37%, Amoxicillin/Clavulanic acid is 36.92%, Ciprofloxacin is 69.69%, Trimethoprim/Sulfamethoxazole 74.24% for *E coli* has been seen.
11. To conclude; most patients with uncomplicated UTI are clinically straight forward and they may not require any laboratory testing beyond urine analysis, but in case of patients with complicated UTI or recurrent UTI or patient with instrumentation laboratory tests are necessary to make diagnosis and to provide specific information regarding identity, antimicrobial susceptibility and to investigate patient outcomes related to biofilm-related infection. So as to necessary control measure can be taken.

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