CHAPTER-I

1. INTRODUCTION

Despite improved methods of prevention and prophylaxis, infection remains one of the most important causes of postoperative morbidity and mortality (Strabelli et al., 2008). Impaired host defense mechanisms following major surgery or trauma are considered important for the development of sepsis (Hensler et al., 1997). Cardiac surgery is associated with high rate of postoperative complications (Bhatia et al., 2003) with renal dysfunction being a persistent complication of cardiopulmonary bypass that when sufficient to require dialysis, increase mortality by eight fold (Rinder et al., 2003; Salenger et al., 2003).The varying degrees of renal dysfunction, impaired humoral and cellular immune response including transient defects in phagocytic function (Silva et al., 1994) and impaired T-cell reactivity (De Angeli et al.,1994) as well as reduction in circulating complement levels (Parker et al., 1972) in patients undergoing cardiopulmonary bypass may be associated with increased susceptibility to infections and sepsis (Thakar et al., 2003).

Patients undergoing cardiac surgery appear to be at increased risk for the development of nosocomial infections (Bonza et al., 2006) due to the presence of multiple surgical wounds (chest and lower extremity incisions), frequent postoperative utilization of invasive devices (i.e. central venous catheters, chest drains, pulmonary artery catheter), and the common use of prophylactic or empiric antibiotics in the perioperative period (Kollef et al., 1997). Postoperative infection has been reported to occur in 5-21% of cardiac surgery patients (Michalopoulos et al., 2006). A report on the 14th European Congress of Clinical Microbiology and Infectious Diseases (2004) mentioned the incidence of microbiologically confirmed infection among patients undergoing cardiac surgery to be 16.2%; sepsis being the most frequent infection (30.3%) followed by catheter (23.4%), sternal wound infection (16.6%), UTI (16.6%) and respiratory tract infection (12.8%).

Although wide experience has been achieved in dealing with these complications, the actual influence of different risk factors has rarely been evaluated (Rebollo et al., 1996). It is therefore imperative to implement adequate measures to diagnose and reduce the incidence of postoperative infections.

Urinary tract infections (UTIs) are one of the most common nosocomial infection (Chandrashekhar et al., 2006, Stamm, 1983).The daily incidence of bacteriuria in catheterized patients is approximately 3-10%. Among patients with bacteriuria, upto 25% will develop symptoms of UTI and about 3% will develop bacteremia (Tenke et al., 2008).The pathogens causing UTIs are almost always predictable with *E. coli* being the primary etiologic agent. Other *Enterobacteriaceae* such as *Klebsiella pneumoniae* and other organisms including coagulase negative staphylococci, *Enterococcus* spp. and group B streptococci, *Pseudomonas aeruginosa* and urease producing organisms such as *Proteus mirabilis*, *Providencia stuartii* and *Morganella morganii* are common as well (Zhanel et al., 2000).

Asymptomatic bacteriuria is the isolation of a specified quantitative count of bacteria in an appropriately collected urine specimen obtained from a person without symptoms or signs referable to urinary tract infection. Asymptomatic bacteriuria is common but its prevalence varies widely with age, gender and the presence of genitourinary abnormalities or underlying diseases (Nicolle et al., 2005). The prevalence of bacteriuria increases with age in healthy women from as low as 1% among school girls to ≥20% among women 80 years of age or greater (Hancock et al., 2008).

Prophylactic intravenous antibiotics should be routinely administered to patients undergoing cardiac surgery. Prolonged administration of antibiotics carries the distinct possibility of suppression of endogenous micro flora, permitting the overgrowth of pathogenic strains, development or selection of antimicrobial agent resistant strains and disruption of colonization resistance (Edwards et al., 2006). The ongoing nature of resistance emergence and dissemination dictates that reliable laboratory procedures be used to detect resistance as an aid to managing the infected patient and as a means of monitoring changing resistance trends among clinically relevant bacteria (Hugo and Russell, 1993).

Though considerable studies involving symptomatic UTI have been done, published data on asymptomatic bacteriuria in postoperative cardiac patients are scarce or non existent in Nepal. Therefore, this study was undertaken to identify the bacterial pathogens from midstream urine samples, to determine the prevalence of asymptomatic bacteriuria among the postoperative patients undergone open heart surgery and also to determine the antibiotic susceptibility pattern of the isolates.

CHAPTER-II

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

To study the bacteriological profile of urine of postoperative patients undergone open heart surgery at Shahid Gangalal National Heart Centre.

2.2 SPECIFIC OBJECTIVES

- To isolate and identify the bacterial pathogens from mid stream urine samples collected from postoperative patients.
- To determine the prevalence of asymptomatic bacteriuria among the postoperative patients who had undergone open heart surgery.
- To determine antibiotic susceptibility pattern of the isolates
- To determine the prevalence of multi drug resistant (MDR) strains among the total isolates.

CHAPTER-III

3. LITERATURE REVIEW

3.1 OPEN HEART SURGERY AND POSTOPERATIVE COMPLICATIONS

Open heart surgery is a surgery in which the patient chest is opened and surgery is performed on the heart. The term "open" refers to the chest, not to the heart itself. The heart may or may not be opened depending on the particular type of surgery. In Nepal, 1279 cardiac surgeries (Congenital (33.33%), valve (32.32%), vascular (22.22%), coronary (11.11%) and others (2.2%) were performed in 2007 in Shahid Gangalal National Heart Centre, a tertiary referral centre for heart patients (SGNHC Annual report, 2007).Despite rapid advancement in surgical techniques, postoperative infections in cardiac surgery remain as dreaded complications (Segers et al., 2006) including bloodstream infection, ventilator associated pneumonia, endocarditis of prosthetic valves, mediastinitis and osteomyelitis (Kothari and Panigrahi, 2001). Patients undergoing cardiac operations are particularly susceptible to infection owing to numerous predisposing factors, such as prolonged operating time, multiplicity of invasive procedures, postoperative intensive manipulation, and high dependency on hospital staff, and the infections are often severe (Rebollo et al., 1996). Postoperative infection has been reported to occur in 5-21% of cardiac surgery patients (Michalopoulos et al., 2006). Ploeg et al., 2007 found infection ratio of 10% (surgical site infection (55.2%), UTI (16.4%), pneumonia (14.9%) and bacteremia (10.4%)) among patients undergoing arterial bypass surgery. Pulmonary infection (15.3%) was the most frequent among the infectious complications in postoperative cardiac patients, followed by bloodstream infection (3.1%), chest surgical wound infection (2.7%) and lower limb infection (2.7%) and urinary tract infection (2.4%) (Strabelli et al., 2008). Cardiothoracic surgery is associated with high rates of postoperative infections which increase the length of hospital stay and cost of treatment as well as increases the mortality (Bhatia et al., 2003).

3.2 PHYSIOLOGICAL CHANGES IN THE KIDNEY AND URINARY TRACT AFTER OPEN HEART SURGERY

The kidney is uniquely vulnerable to injury as a result of its anatomy and physiology (Yallop and Smith, 2004) and renal dysfunction occurs commonly after cardiac operations (Salenger et al., 2003). The patient undergoing a major surgical procedure is at risk for the development of postoperative acute renal failure (ARF) (Boldt and Wolf, 2008). This is especially true for cardiac operations, which are considered to involve more risk than other types of operations because of the use of extracorporeal circulation and the higher likelihood of cardiovascular instability during and after the intervention (Zanardo et al., 1994, Rosner and Okusa, 2006).ARF is a serious complication of cardiac surgery causing high morbidity and mortality (Bove et al., 2005). ARF occurs in up to 30% of patients who undergo cardiac surgery, with dialysis being required in approximately 1% to 5% of all patients (Chukwuemeka et al., 2005).

Similarly, patients undergoing cardiopulmonary bypass may have impairment to the humoral and cellular immune response. Silva et al., 1994 and De Angeli et al.,1994 reported transient defects in phagocytic function and impaired T-cell reactivity, respectively, associated with cardiopulmonary bypass procedures. In a similar study, Parker et al., 1972 demonstrated a reduction in circulating complement levels in patients undergoing cardiopulmonary bypass. These data raise the question that varying degrees of renal dysfunction and related metabolic/cellular events, as well as certain intraoperative factors, may be associated with increased susceptibility to infections and sepsis (Thakar et al., 2003).

3.3 URINARY TRACT INFECTION (UTI) AS A NOSOCOMIAL INFECTION

The NNIS (National Nosocomial Infection Surveillance) system defines a nosocomial infection as a localized or systemic condition that results from adverse reaction to the presence of an infectious agents (or its toxins) and that was not present or incubating at the time of admission to the hospital (Garner et al., 1996). Nosocomial infections may be either endogenous or exogenous. Endogenous infections are caused by those organisms that are present as a part of normal flora of the patient (autoinfection) (Haley et al., 1985); exogenous infections are those acquired by exposure to hospital personnel (Maki, 1978), medical devices (Hsueh et al., 2002) or the hospital environment (Cookson, 2005).

Nosocomial infections after open heart surgery are recognized as an important cause of mortality, morbidity, prolonged hospital stay, increased need for antimicrobial therapy and higher concomitant costs (Guardia et al., 2008; Bonza et al., 2006; Akcam et al., 2006). The reported prevalence for nosocomial infections after open heart surgery (Segers et al 2006; Michalopoulos et al., 2006) most commonly ranges from 5 to 20%, but can be significantly greater among patients requiring intensive care.

In the cardiac surgical postoperative period, nosocomial infections have been found to be associated with prolonged length of stay in the ICU and total hospitalization, development of multiorgan dysfunction, and increased hospital mortality.The use of urinary and central venous catheters increases the likelihood of a nosocomial infection approximately sevenfold (Glynn and Sheehan, 1984). Michalopoulos et al., 2006 reported three risk factors to be independently associated with nosocomial infection after open heart surgery, namely; history of immunosuppression, transfusion of more than five red blood cell units during the first postoperative day in both operating room and ICU, and development of acute renal failure during the first two postoperative days.

The urinary tract is the most common site of nosocomial infection (Stamm, 1983) accounting for approximately 40% of nosocomial infections (Tenke et al., 2008). Nosocomial UTI is defined as the occurrence of a UTI, at least 48 hours after a hospital admission, with an isolation of at least 10^5 cfu / ml in culture (Stark and Maki, 1984; Sen et al., 2006). The CDC definitions stratify nosocomial UTI into symptomatic, asymptomatic and other infections of urinary tract (Garner et al., 1996).

3.4 DEFINITION OF UTI

The urinary tract is the second most common site of bacterial infection in human (Leigh, 1990). UTI encompasses a wide range of clinical entities whose common denometer is microbial invasion of any tissue of the tract from renal cortex to the urethral meatus (Singh, 1991). Infection of the prostate and epididymis is also included in the definition but not urethritis caused by gonococci and chlamydiae because of their unique characters and strict localization to the urethra and genital system. In order to confirm UTI with reasonable confidence, the criteria of clinical features, bacteriuria and pyuria must be met. The presence of bacteria in urine is called bacteriuria (Cheesbrough, 1984). Significant bacteriuria indicates that organisms are actually multiplying in the urine and present in a count, which is excessively higher or unexplainable by urethral contamination (Pokhrel, 2004).

3.5 CLASSIFICATION OF UTI

The clinical presentation of UTIs may vary, ranging from asymptomatic infection to full- blown pyelonephritis.

Urethritis (infection of urethra) is characterized by dysuria and frequency. *Chlamydia trachomatis, Neisseria gonorrhoeae* and *Trichomonas vaginalis* are common causes of urethritis and are considered to be sexually transmitted.

Typically, patients with **cystitis** (infection of the bladder) complain of dysuria, frequency and urgency. These symptoms are due not only to inflammation of the bladder but also to multiplication of bacteria in the urine and urethra.

Another UTI is **acute urethral syndrome**. Patients with this syndrome are primarily young, sexually active women, who experience dysuria, frequency and urgency but yield fewer organisms than 10^5 cfu/ml on culture. Although *Chlamydia trachomatis* and *N. gonorrhoeae* urethritis, anaerobic infection, genital herpes and vaginitis account for some cases of acute urethral syndrome, most of these women are infected with organisms identical to those that cause cystitis but in numbers less than 10^5 cfu/ml urine.

Pyelonephritis refers to inflammation of the kidney parenchyma, calices and pelvis and is usually caused by bacterial infection. The typical clinical presentation of an upper UTI includes fever and flank (lower back) pain and frequently, lower tract symptoms (frequency, urgency and dysuria) (Forbes et al., 2007).

Asymptomatic significant bacteriuria has variously been termed symptomless or covert bacteriuria (Leigh, 1990) or asymptomatic UTI (Forbes et al., 2007).The definition of asymptomatic bacteriuria is controversial as some have defined it as the quantitative growth of bacteria, greater than or equal to 10^5 cfu/ml urine of the same organism, on aseptically collected midstream urine specimens, in the absence of symptoms of urinary tract infection on two or more consecutive occasions (Zdziarski et al., 2008; Harding et al., 2002) while for others a single occasion is sufficient (Matteucci et al., 2007 Cumming et al., 2006, Akcam et al., 2006; Gleckman et al., 1979).

According to the IDSA (Infectious Diseases Society of America) guidelines for asymptomatic women, bacteriuria is defined as 2 consecutive voided urine specimens with isolation of the same bacterial strain in quantitative counts $\geq 10^5$ cfu/mL. A single, clean-catch voided urine specimen with one bacterial species isolated in a quantitative count $\geq 10^5$ cfu/ml identifies bacteriuria in men. A single catheterized urine specimen with one bacterial species isolated in a quantitative count $\geq 10^2$ cfu/ml identifies bacteriuria in women or men (Nicolle et al., 2005).

3.6 EPIDEMIOLOGY AND MICROBIOLOGY OF URINARY TRACT INFECTION

The prevalence of UTI is dependent on a number of factors and those precipitating the development of UTI include anatomical (congenital abnormalities, prostatic hypertrophy, cystocele, uterine prolaspe), pathological (surgical operations on the urogenital tract, tumours of bladder and prostate, urethral catheterization, atrophic vaginitis, neurological disorders of bladder), infective (vulvovaginitis, vaginal discharge), social (sexual intercourse, menstruation, sanitary pads, intrauterine coil) and environmental (nylonwear, tight clothes, long distance driving) factors (Leigh, 1990). Symptomatic UTI comprised 16.4% of the infections among patients undergoing arterial bypass surgery (Pleog et al., 2007). Strabelli et al., 2008 reported the prevalence of symptomatic UTI among postoperative cardiac patients to be 2.4%.

The prevalence of asymptomatic bacteriuria (ABU) is higher among boys (2.5%) than girls (0.9%) during infancy but after infancy, it is much higher among girls (1% to 2%) than among boys (<0.1%) (Jha and Singh, 2007). The presence of bacteriuria in neonates and infants is an indication for investigation to rule out congenital malformations, especially vesicoureteral reflux that can produce renal scars that are responsible for renal insufficiency and hypertension several years later (Raz, 2003). Women identified with asymptomatic bacteriuria in early pregnancy have a 20–30-fold increased risk of developing pyelonephritis during pregnancy, compared with women without bacteriuria. These women also are more likely to experience premature delivery and to have infants of low birth weight (Nicolle et al., 2005). Women with diabetes have higher risks of UTI and asymptomatic bacteriuria (Ribera et al., 2005).The incidence of catheter-associated bacteriuria is related to the type and duration of catheterization, the health-care personnel inserting the catheter, the catheter system and care. A short-term catheterization causes bacteriuria in 1 - 5% of patients. In elderly patients with indwelling catheters draining in an open system, the incidence of bacteriuria is almost 100% and approximately 20% in a closed system for up to 14 days (Raz, 2003).

Table 1.Prevalence of asymptomatic bacteriuria in selected populations (Nicolle et al., 2005)

Population	**Prevalence (%)**
Healthy, premenopausal women	1.0–5.0
Pregnant women	1.9–9.5
Postmenopausal women aged 50–70 years	2.8–8.6
Diabetic patients	
Women	9.0–27
Men	0.7–11
Elderly persons in the community	
Women	10.8–16
Men	3.6–19
Elderly persons in a long-term care facility	
Women	25–50
Men	15–40
Patients with spinal cord injuries	23–89
Intermittent catheter use	
Sphincterotomy and condom catheter in place	57
Patients undergoing hemodialysis	28
Patients with indwelling catheter use	
Short-term	9–23
Long-term	100

Escherichia coli remains the most common organism isolated from bacteriuric women. Other *Enterobacteriaceae* (such as *Klebsiella pneumoniae*) and other organisms (including coagulase-negative staphylococci, *Enterococcus* species, group B streptococci, and *Gardnerella vaginalis*) are common as well. For men, coagulase-negative staphylococci are also common, in addition to gram-negative bacilli and *Enterococcus* species (Nicolle et al., 2005). Organisms isolated in patients with asymptomatic bacteriuria will be influenced by patient variables; healthy persons will likely have *E. coli*, whereas a nursing home resident with a catheter is more likely to have multi-drug resistant polymicrobic flora e.g., *Pseudomonas aeruginosa* and urease-producing organisms such as *Proteus mirabilis*, *Providencia stuartii*, and *Morganella morganii* (Colgan et al., 2006) .

Escherichia coli 83972 is a prototype ABU strain and undoubtedly the best-characterized ABU-class *E. coli* to date. Strain 83972 was originally isolated in the 1970s from a young Swedish girl who had carried it for at least 3 years without symptoms. The strain is well adapted for growth in the human urinary tract where it establishes long-term bacteriuria (Hancock et al., 2008). The strain lacks defined O and K surface antigens and is nonmotile. Also, electron microscopy of 83972 did not reveal the presence of flagella or a capsule (Klemm et al., 2007).

Jha and Singh, 2007 reported asymptomatic bacteriuria in 1.39% of school going children in Pokhara valley and found *Escherichia coli* (57.14%) to be the predominant bacterial pathogen.

Cumming et al., 2006 reported that the prevalence of asymptomatic bacteriuria in sickle cell disease was 10.9%. *E. coli* (55%) was the commonest organism. Other urinary tract pathogens identified in this study included group B *Streptococcus*, *Enterobacter aerogenes*, coagulase negative staphylococci, *Enterobacter cloacae*, *Proteus mirabilis*, and *Bacteroides*, *Streptococcus viridans*, group D *Streptococcus*, *Klebsiella pneumoniae* and *Enterobacter koseri*.

Turan et al., 2008 found asymptomatic bacteriuria in 17.8% of type 2 diabetes mellitus patients and the most common pathogen in patients with asymptomatic bacteriuria was *E. coli* (68%) followed by *Klebsiella pneumoniae* (23%), *Enterobacter aerogenes* (4.5%) and group B β haemolytic streptococci (4.5%).

The most common microorganism isolated among the institutionalized Chinese elderly was *Escherichia coli* (29.7%) followed by *Klebsiella pneumoniae* (21.6 %), *Providencia stuartii* (16.2%), and *Pseudomonas aeruginosa* (13.5%) (Lin et al., 2006).

Kumar et al., 2002 observed asymptomatic bacteriuria in 10.57% of school going children with female preponderance over male. The maximum isolates were *E .coli*

(32.8%) followed by *Klebsiella pneumoniae* (22.4%) and *Staphylococcus aureus* (15.1%).

E. coli were isolated in 18% of cases, coagulase positive staphylococci in 30%, coagulase negative staphylococci in 15%, *Streptococcus pyogenes* in 6%, *Klebsiella* species in 9% and enterococci in 21% of cases (el-Gamal and Saleh ,1991).

Vaishnavi et al., 2004 reported that forty four (12.6%) of the patients (18 symptomatic; 26 asymptomatic) with gallbladder diseases showed bacteriuria and concluded UTI as a frequent concomitant of gall bladder diseases.

A study conducted by Ullah et al., 2007 showed the prevalence of asymptomatic bacteriuria to be 12% among the pregnant mothers in rural Rajshahi.

Hermida and Ferrer, 2004 found that the prevalence of asymptomatic bacteriuria was 6.34% with *E. coli* (77.87%) being the most frequently isolated. A statistically significant greater number of coronary artery disease, other heart diseases, and cardiovascular disorders were found among patients with asymptomatic bacteriuria in comparison with non-asymptomatic bacteriuria controls.

3.7 PATHOGENESIS

Whether an organism is able to colonize and then cause UTI is determined in large part by a complex interplay of host and microbial factors.

3.7.1 Route of infection

Bacteria can invade and cause a UTI via ascending, haematogenous or lymphatic routes. Although the ascending route is the most common route of infection in females, ascent in association with instrumentation (eg urinary catheterization, cytoscopy) is the most common cause of nosocomial UTIs in both sexes. For UTIs to occur by the ascending pathway, gram negative enteric bacteria and other microorganisms that colonize the gastrointestinal tract must be able to colonize the vaginal cavity and/ or the periurethral area. Once these organisms gain access to the bladder, they must multiply and then pass up the ureters to the kidneys (Foxman and Brown, 2003).

Haematogenous spread which accounts for less than 5% of UTIs occurs as a result of bacteremia. Any systemic infection can lead to seeding of the kidney but certain organisms eg *Staphylococcus aureus* or *Salmonella* spp. are particularly invasive. Yeast (usually *Candida albicans*), *Mycobacterium tuberculosis, Salmonella* spp., *Leptospira* spp. or *Staphylococcus aureus* in the urine often indicates pyelonephritis acquired via haematogenous (bloodborne) spread or descending route (Kunin, 1994).

Lymphatic route results in UTI when there is direct extension of bacteria from the adjacent organs via lymphatics. This may occur in unusual circumstances, such as severe bowel infection or retroperitoneal abscess and there is little evidence that this route plays a significant role in the vast majority of UTIs (Schaeffer, 1998).

3.7.2 Host defense mechanisms of the urinary tract

Urine excreted in the kidney is sterile unless the kidney is infected. Uncontaminated bladder urine is also normally sterile (Brooks et al., 2004) but the proximity of the urethral meatus to the anus means that, particularly in women, small numbers of potentially pathogenic organisms are constantly entering the urethra and bladder (Leigh, 1990).

Although the urethra hosts a resident microflora that colonizes its transitional epithelium consisting of coagulase negative staphylococci, viridans and non haemolytic streptococci, lactobacilli, diptheroids, non pathogenic *Neisseria* species, transient gram negative aerobic bacilli(including Enterobacteriaceae), anaerobic cocci, *Propionibacterium* species, anaerobic gram negative cocci and bacilli, commensal *Mycobacterium* species, commensal *Mycoplasma* species and occasional yeasts, all area of the urinary tract above the urethra in a healthy human are sterile (Forbes et al., 2007).

A number of defense mechanisms in the urinary tract contribute to its resistance to infection. Urine itself is inhibitory to some of the urethral flora such as anaerobes. In addition, if urine has a low pH, high or low osmolality, high urea concentration or high organic acid content, even organisms that can grow in the urine may be inhibited. If bacteria do gain access to the bladder, the constant flushing of contaminated urine from the body either eliminates bacteria or maintains their numbers at low levels. Any interference with the act of normal voiding such as mechanical obstruction resulting from kidney stones or strictures will promote the development of UTI .The impermeability of the uroepithelium to bacterial invasion is the other most critical component of defence mechanism. A number of factors appear to inhibit the adherence of bacteria to the uroepithelium. The glycosaminoglycans component of the luminal transitional cell surface is extremely hydrophilic and may prevent bacterial attachment (Parsons and Mulholland, 1978). Tamm-Horsfall protein, which is the most abundant urinary protein of renal origin, binds with type 1 adhesins of *E. coli*. This leads to bacterial agglutination and may block adhesion- mediated adherence of the vesicle mucosa. Each of these events would promote clearance of bacteria during micturation. Bacterial- specific urinary antibody of the IgG and IgA classes also appear to inhibit bacterial adherence (Fowler and Mariano, 1984).

A zinc containing polypeptide referred to as prostatic antibacterial factor or PAF appears to be the most significant antimicrobial constituent of prostatic fluid (Fair and Wehner, 1976). In addition to bactericidal activity against gram negative bacilli, the substance inhibits the growth of viruses (Fridlender et al., 1978), yeast, trichomonands (Krieger and Rein, 1982) and *Chlamydia trachomatis* (Mardh et al., 1980). Prostatic fluid is also rich in spermine, which has some activity against gram positive bacteria. A remarkable local immune response of the prostate to bacteriuria has been demonstrated (Fowler and Mariano, 1982).

A possible natural antibiotic called human beta defensin-1 (HBD-1) fights *E. coli* within the female urinary and reproductive tract. Research suggests that when bacteria infect the bladder, the cells that line the bladder literally sacrifice themselves and self- destruct (a process called apoptosis) i.e they shed away from the lining, carrying the bacteria with them. This eliminates about 90% of *E. coli* (Mulvey et al., 2000). The increased length of the male urethra and a washout of bacteria during micturation have also been suggested as important defence mechanism (Fowler and Mariano, 1984).

Despite various defense mechanisms of the human urinary tract, there is dynamic culture system in the urinary tract in which bacteria undergo multiplication while urine is continuously being added by glomerular filtration and lost by micturation. The bacterial population will be controlled by its growth rate and the balance between the speed of the urinary flow and the volume of the system.

Components that support bacterial growth are urinary pH, osmolality and chemical constituents such as glucose and amino acids etc. Normal urine usually contains sufficient glucose to support maximum growth rates and any lowering of the pH is prevented by its buffering capacity. Glucose provides sources of energy for the growth of urinary pathogenic bacteria. The number of bacteria in the urine of diabetic patients was significantly higher than in that of non diabetic controls due to high level of glucose (Leigh, 1990).

3.7.3 Bacterial virulence factors for the establishment of infection

The ability of UPEC (Uropathogenic *E. coli*) to cause symptomatic UTI is enhanced by adhesins (eg type 1 and P fimbriae) and toxins (eg haemolysin) (Brzuszkiewicz et al., 2006; Klemm and Schembri, 2000). P fimbriae bind to the α-D-galactopyranosyl-(1-4)-β-D-galactopyranoside receptor epitopes in the globoseries of glycolipids Similarly, type 1 fimbriae confer binding to α -D-mannosylated proteins such as uroplakins, which are abundant in the uroepithelial lining of the bladder (Wu et al., 1996). Both P- and type 1 fimbriae recognise their receptor targets by virtue of organelle tip-located adhesins, namely PapG and FimH, respectively (Klemm and Schembri, 2000). UPEC also express K (capsular) antigens which are antiphagocytic and are partly responsible for serum resistance. UPEC usually produce siderophores which play a role in iron acquisition for the bacteria during and after colonization and haemolysins eg aerobactin which are cytotoxic. Uropathogens, particularly *Proteus mirabilis and P. vulgaris* and also *Klebsiella* spp. and *Staphylococcus saprophyticus* produce urease that hydrolyze urea. This results in the increase in urine pH that is directly toxic to kidney cells and also stimulates kidney stone formation. Motility may be important for the organisms to ascend to the upper urinary tract against the flow of urine and cause pyelonephritis.

The pathogenesis of asymptomatic bacteriuria (ABU) is different from that of symptomatic UTI. ABU patients may carry a single strain for months or years, creating a condition that resembles commensalism, but with a strain that may have evolved from a pathogenic ancestor (Hancock and Klemm, 2007). The clinically benign nature of ABU was initially explained by a lack of virulence, since phenotypically many ABU *E. coli* strains are non-hemolytic, non adherent and lack defined serotype markers (Mabbett et al., 2008). Molecular epidemiology revealed, however, that > 60% of ABU strains carry virulence genes, even though they fail to express the corresponding phenotype (Klemm et al., 2007). For example, 60% of ABU strains were pap DNA positive, but less than 20% of those strains expressed P fimbriae, suggesting that ABU strains may have arisen from virulent UPEC strains but achieved long-term persistence by attenuation of virulence factors that provoke a host response (Zdziarski et al., 2008).
E. coli 83972, a prototype ABU strain, avoids triggering host defense mechanisms, lives a commensal- like existence in bladder urine, and does not ascend further than the bladder (Klemm et al., 2007). In contrast to organisms that have acquired genes for pathogenesis, *E. coli* 83972 is an example of an organism that has adapted to a commensal-like existence through gene deletions and point mutations (Hancock et al., 2008).

The human urinary tract is submitted to significant hydrodynamic shear forces, and fimbriae-mediated adherence to the urinary tract epithelium is generally believed to be important for the bacteria to resist removal by urine flow and to establish in this niche (Roos et al., 2006a). The *E. coli* 83972 strain lost the ability to express functional UPEC class fimbriae, probably as an evolutionary tradeoff with the host defense. This ensured that it did not attract the attention of aggressive host defense mechanisms, such as cytokine production, inflammation, and exfoliation of infected bladder cells. However, in order to avoid being flushed out of the system, it had to adapt to a particular ecological niche, i.e., human urine as a growth medium, and to optimize its growth rate to keep pace with the flow rate in the bladder (Klemm et al., 2007).

The ability of ABU strains to establish in the human bladder is first and foremost due to its excellent growth properties in human urine. Strain 83972 grows to higher maximum cell densities and exhibits a shorter lag phase than any of the UPEC strains (Roos et al., 2006b; Klemm et al., 2007). The excellent biofilm-forming faculty of strain 83972 might also contribute to its ability to outcompete other microbes and to inhibit urinary tract infection and catheter colonization by uropathogens (Klemm et al., 2007). The ABU strains may bind to a class of receptors that do not participate in signaling and host cell activation, thus benefiting from adherence without provoking the antibacterial defence. This may constitute a mechanism by which some ABU *E. coli* evade normal immune surveillance and persist in the bladder (Mabbett et al., 2008).

The ABU strain has adapted well to grow in the iron-limiting environment in the urine by up regulation of its numerous iron uptake systems including production of siderophores such as aerobactin and enterobactin. Furthermore, there is high expression levels of genes involved in transportation and degradation pathways of sugar acids and carbohydrates (e.g., galacturonate, glucuronate, galactonate, arabinose, sorbitol, galactose, maltose, xylose, and mannose) (Klemm and Schembri , 2000).

3.8 DIAGNOSIS OF ASYMPTOMATIC BACTERIURIA

3.8.1 Methods of specimen collection and transport

Prevention of contamination by normal vaginal, perineal and urethral flora is the most important consideration for collection of a clinically relevant urine specimen. Various methods for the collection of urine specimens are available including clean catch midstream urine, straight catheterized urine specimen, suprapubic bladder aspiration, indwelling catheter, etc. Although slightly more invasive, urinary catheterization may allow collection of bladder urine with less urethral contamination. Risk exists, however, that urethral organisms will be introduced into the bladder with the catheter. Either a physician or other trained health professionals perform this procedure. In suprapubic bladder aspiration**,** urine is withdrawn directly through a percutaneously inserted needle, thereby ensuring a contamination free specimen. The bladder must be full before performing the procedure. If good aseptic techniques are used, this procedure can be performed with little risk in premature infants, infants, small children and pregnant women and other adults with full bladders. Specimen collection from patients with indwelling catheters requires scrupulous aseptic technique. The catheter tubing should be clamped off above the port to allow collection of freshly voided urine. In catheterized patients, urine should be collected directly from the catheter and not from the collection bag because organisms can multiply there, obscuring the true relative numbers

The least invasive procedure, the clean- catch midstream urine specimen collection must be performed carefully for optimal results, especially in females. Female patients should be instructed to cleanse the area around the urethral opening with clean water, dry the area and begin to void and then collect 10-20 ml of urine in dry, wide necked, leak proof sterile container. If the patient is in renal failure or a young child, it may not be possible to obtain more than a few milliliters. Since the urine stream flushes out the potentially contaminating urethral flora, midstream urine is the specimen of choice. Whenever possible, the first urine passed by the patient at the beginning of the day should be sent for examination. This specimen is the most concentrated and therefore the most suitable for culture, microscopy and biochemistry. The container labeled with the date, the name and number of the patient and time of collection should be immediately delivered to the laboratory (Cheesbrough, 1984). Following changes may occur when unpreserved urine is left at room temperature:

- Any bacteria in the urine will multiply so that bacterial count will be unreliable. If organisms are urease producing, the ammonia released will increase the pH of the specimen which will result in the destruction of cells and casts. Bacteria will also breakdown any glucose which may be present.
- If white cells, red cells and casts are present, these will begin to lyze especially in a concentrated specimen.
- The concentration of protein in the urine will be altered. If bilirubin is present, this may be oxidized to biliverdin which will not be detected. Likewise, urobilinogen will not be detected because it will be oxidized to urobilin (Cheesbrough, 1984).

Since urine is an excellent supportive medium for growth of most bacteria, urine should be refrigerated at 4^0C, if immediate transportation to the laboratory is not possible. But even with refrigeration, some species may grow and leucocytes, erythrocytes and casts may become degraded. If a delay in delivery of more than 1 hour is anticipated, 1.8% boric acid should be added to the urine. Specimens containing boric acid need not be refrigerated (Leigh, 1990). But leucocytes are rapidly degraded in such specimens. Alternately, Nacl, polyvinylpyrolidine may also be used. Urine for culture must not be preserved with a bactericidal chemical such as thymol, bleach, HCl, acetic acid or chloroform (Cheesbrough, 1984). If unpreserved samples are delayed more than 5 hours in transit to the laboratory, the doctor should be informed and samples discarded for positive findings may be misleading (Collee et al., 1996).

3.8.2 Urine culture

The count of viable bacteria in a correctly collected sample of urine is the gold standard for the diagnosis of bacteriuria. The most accurate methods of counting bacteria are the pour plate and the surface viable count but they are time consuming and for routine purposes semiquantitative techniques are used. These are useful for examining large numbers of urine specimens; the most commonly used techniques are the standard loop, filter paper strip, dip spoon and dip slide (Leigh, 1990).

Standard loop method: An inoculating loop of standard dimensions is used to take up a small, approximately fixed and known volume of mixed uncentrifuged urine and is inoculated onto an agar culture medium. The sterile standard calibrated loop is inserted vertically into the thoroughly mixed urine in the container. Otherwise, more than the desired volume of urine will be taken up, potentially affecting the quantitative culture result. If the urine is in a small diameter tube, the surface tension will alter the amount of specimen picked up by the loop. The choice of which media to inoculate depends on the patient population served and the microbiologist's preference. The use of a 5% sheep blood agar plate and a MacConkey agar plate allow detection of most gram-negative bacilli, staphylococci, streptococci and enterococci (Forbes et al., 2007). Instead of using two media, some laboratory prefer CLED (Cystine Lactose Electrolyte Deficient) medium (Leigh, 1990).

Once plated, urine cultures are incubated overnight at 35^{o}C. For the most part, incubation for a minimum of 24 hours is necessary to detect uropathogens. Thus, some specimens inoculated late in the day cannot be read accurately the next morning. These cultures should either be reincubated until the next day or possibly interpreted later in the day when a full 24 hour incubation has been completed (Forbes et al., 2007).

Interpretation of urine cultures

According to Kass, Marple and Stanford criteria to interpret significant bacteriuria, bacterial count of less than 10^5 cfu/ml corresponds to contaminants whereas that equal to or more than 10^5 cfu/ml corresponds to significant bacteriuria. If this number of organisms is found in a single urine sample from a given individual, the probability of true bacteriuria is about 80% and if the observation is repeated, the probability rises to about 96% (Leigh, 1990).

A count of 10^4 to 10^5 cfu/ml indicates low count significant bacteriuria which is subject to following conditions (Pokhrel, 2004).

1. Urine was collected before the organisms reached to log phase of growth after the entry of bacteria into the urinary tract.
2. Patient under treatment.
3. Sometimes in younger female, the count is low such as honey moon cystitis.
4. Patient with certain endocrine disorder e.g. Diabetes
5. Chronic kidney infection where concentration power of kidney is low.
6. Obstruction in the ureters.
7. Infection with relatively slow growing organisms e.g. *Staphylococcus epidermidis*, streptococci other than enterococci, *Haemophilus influenzae.*

A pure culture of *Staphylococcus aureus* is considered to be significant regardless of number of cfus and antibiotic susceptibility testing are performed. The presence of yeasts in any number is reported to physician and pure cultures of yeasts may be identified to the species level (Forbes et al., 2007).

3.9 ANTIMICROBIAL SUSCEPTIBILITY TESTING

The primary goal of antimicrobial susceptibility testing is to determine whether the bacterial etiology of concern is capable of expressing resistance to the antimicrobial agents that are potential choices for therapy. Since therapy of infection begins before laboratory results are available, antibiotic susceptibility testing primarily plays a supplementary role in confirming that the organism is susceptible to the agent that is being used. Sometimes it may enable the clinician to change from a toxic to a less toxic agent or from an expensive to a cheaper one (Greenwood et al., 2000).

Kirby- Bauer disc diffusion method is used by most laboratories to test routinely for antimicrobial susceptibility testing. Using this test, antimicrobial resistance is detected by allowing the antibiotics to diffuse from a point source, commonly in the form of an impregnated filter paper disc, into an agar medium that has been seeded with the test organism. Visible growth of bacteria occurs on the surface of the agar where the concentration of antibiotic has fallen below its inhibitory level for the test strain (Collee et al., 1996). Following incubation, the diameter of the zone of inhibition around each disc is measured in millimeters and reported as susceptible, intermediate or resistant. However, the laboratory test conditions in no way mimic the in vivo environment at the infection sites where the antimicrobial agents and bacteria will actually interact.

3.10 SCREENING AND TREATMENT OF ASYMPTOMATIC BACTERIURIA

Although persons with bacteriuria are at an increased risk of asymptomatic urinary infection, treatment of asymptomatic bacteriuria doesn’t decrease the frequency of symptomatic infection or improve other outcome. Screening and treatment of asymptomatic bacteriuria has been recommended only for pregnant women, males undergoing transurethral resection of prostate and individuals undergoing urological procedures for which mucosal bleeding is anticipated. Guidelines published by the IDSA in 2005 state that there is no measurable benefit to screen for or provide antibiotic treatment of asymptomatic bacteriuria in pre-menopausal non pregnant women, diabetic women, older persons living in the community, older institutionalized subjects, persons with spinal cord injury or catheterized patients while the catheter is in place.

CHAPTER-IV

4. MATERIALS AND METHODS

4.1 MATERIALS

The materials required for this work are listed in Appendix II.

4.2 METHODS

The study was performed prospectively from March to September, 2008 in the laboratory of Shahid Gangalal National Heart Centre, Bansbari, a tertiary referral centre for heart patients in Nepal.

Study population:

Patients of all age groups and both sexes who had undergone open heart surgery and at least 48 hours after removal of urinary catheter were included in the study.

Sample Collection and Transportation (Cheesbrough, 1984):

The patient was instructed to cleanse the area around the urethral opening with clean water, dry the area and begin to void and then collect 10-20 ml of first morning mid-stream urine (MSU) specimen in the provided sterile, dry, wide necked, leak proof container. The container labelled with the date, name and number of patient and time of collection along with the request form was delivered to the laboratory immediately after sample collection.

Culture, Identification and Antibiotic Susceptibility test (Forbes et al., 2007):

The urine specimen was cultured by semi quantitative culture technique. A loopful of mixed, uncentrifuged urine was inoculated on the surface of Blood agar (BA) and MacConkey agar (MA) by streaking with a sterile standard calibrated inoculating loop of internal diameter of 4 mm.

The inoculated MA and BA plates were incubated at 37^0C for 24 hours.

Enumeration of colonies was done and reported as significant bacteriuria ($\geq 10^5$ cfu/ml), doubtful significant (10^4 - 10^5cfu/ml) and insignificant ($<10^4$cfu/ml).

Identification of significant isolates was done by using standard microbiological techniques (Cheesbrough, 1984; Forbes et al., 2007) that included study of colony morphology, Gram staining and biochemical tests (Catalase test, Oxidase test, TSI test, SIM test, Citrate utilization test, Urea hydrolysis test and others as required).

Antibiotic susceptibility test of the identified bacteria was performed by Kirby Bauer disc diffusion method as recommended by NCCLS. The broth culture of test organism with turbidity matched to that of McFarland tube no.0.5 was swabbed uniformly on the surface of Mueller Hinton Agar (MHA).The antibiotic discs were then placed aseptically over the lawn of test organism. After incubation at 37^0C for 18 hours, the diameter of zone of inhibition around each disc was measured, compared with standard chart and then reported as resistant, intermediate or susceptible. Control strains were used in parallel as a part of quality control test systems.

Collection of Midstream urine (MSU) specimen (Cheesbrough, 1984)

↓

Inoculation into Blood Agar and MacConkey Agar by semiquantitative standard loop method

↓

Incubation at $37^{0}C$ for 24 hours

↓

Enumeration of isolated colonies

↓

Significant bacteriuria | insignificant or no growth

Significant bacteriuria ↓

Identification of significant isolates (Cheesbrough, 1984; Forbes et al., 2007)

↓

Antibiotic susceptibility testing by Kirby Bauer disc diffusion method

insignificant or no growth ↓

Report accordingly

Figure 1: Flow diagram for processing urine sample

CHAPTER -V

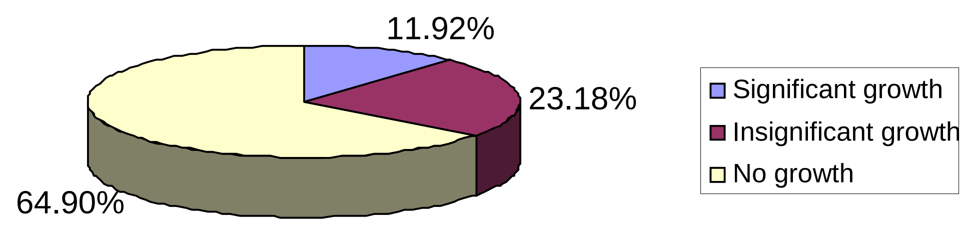
5. RESULTS

This study was conducted among postoperative patients who had undergone open heart surgery at Shahid Gangalal National Heart Centre (SGNHC). One hundred and fifty one midstream urine samples were collected and processed in the microbiology laboratory of SGNHC.

5.1 Pattern of culture results

Out of 151 samples processed for culture, 18 (11.92%) samples showed significant bacteriuria (i.e.>10^5 cfu/ml), 35 (23.18%) samples showed insignificant growth (i.e. ≤10^5 cfu/ml) while 98 (64.90%) samples showed no growth. Among 18 significant bacteriuric cases, 17 were asymptomatic (Figure 2).

Figure 2. Pattern of culture results



5.2 Age and sex wise distribution of significant bacteriuric cases

Among the significant bacteriuric cases, the highest number of cases i.e. 6 belonged to the age group 20-30 years. Females of age group 20-30 were found to have significant bacteriuria in 4 cases (Table 2).

Table 2. Age and sex wise distribution of significant bacteriuric cases

Age group	Male	Female	Total
0-10	1	1	2
10-20	1	2	3
20-30	2	4	**6**
30-40	1	1	2
40-50	0	2	2
50-60	0	0	0
60-70	2	0	2
70-80	0	1	**1**
Total	**7**	**11**	**18**

5.3 Surgery wise distribution of significant bacteriuric cases

The highest percentage of significant bacteriuric cases was observed among valve surgery (15.91%) followed by vascular surgery (10.52 %) (Table 3).

Table 3. Surgery wise distribution of significant bacteriuric cases

Type of surgery	Total no. of surgery	No. of significant bacteriuric cases (%)
Valve	88	14 (15.91)
Vascular	19	2 (10.52)
Congenital	37	2 (5.40)
Miscellaneous	7	0 (0.00)
Total	**151**	**18**

5.4 Pattern of bacterial isolates

Out of 18 significant bacteriuric cases, 14 (77.78%) were due to gram negative bacteria and 4 (22.22%) were due to gram positive bacteria. Six different species of bacteria were isolated among which *Escherichia coli* (44.44%) was found to be the predominant followed by *Klebsiella pneumoniae* (16.67%). The other organisms were *Acinetobacter* spp. (11.11%), *Staphylococcus aureus* (11.11%), *Enterococcus* spp. (11.11%) and *Pseudomonas aeruginosa* (5.56%) (Table 4).

Table 4. Pattern of bacterial isolates

Organisms	**No. of isolates (%)**	**% of total isolates**
Gram positive bacteria		
Staphylococcus aureus	2 (50)	11.11
Enterococcus spp.	2 (50)	11.11
Total	**4 (100)**	**22.22**
Gram negative bacteria		
Escherichia coli	8 (57.14)	44.44
Klebsiella pneumoniae	3 (21.43)	16.67
Acinetobacter spp.	2 (14.29)	11.11
Pseudomonas aeruginosa	1 (7.14)	5.56
Total	**14(100)**	**77.78**

5.5 Surgery wise distribution of bacterial isolates from urine

Out of 8 *E .coli* isolates, the highest number was detected among valve surgery cases. Similarly, all the isolates of *K. pneumoniae, Acinetobacter* spp. and *Pseudomonas aeruginosa* were isolated among valve surgery cases (Table5).

Table 5. Surgery wise distribution of bacterial isolates from urine

Organisms isolated	**Type of surgery**			**Total**
	Valve	Vascular	Congenital	
Escherichia coli	6	1	1	8
Klebsiella pneumoniae	3	0	0	3
Acinetobacter spp	2	0	0	2
Pseudomonas aeruginosa	1	0	0	1
Staphylococcus aureus	1	1	0	2
Enterococcus spp.	1	0	1	2
Total	**14**	**2**	**2**	**18**

5.6 Antibiotic susceptibility pattern of the isolates

5.6.1 Antibiotic susceptibility pattern of the *E. coli* isolates

The antibiotic susceptibility pattern of *E. coli* showed that 100% of the isolates were sensitive to amikacin followed by ceftriaxone with a susceptibility of 75% and nitrofurantoin with a susceptibility of 62.50% (Table 6).

Table6. Antibiotic susceptibility pattern of the *E. coli* isolates (n=8)

Antibiotics used	**Sensitive**		**Intermediate**		**Resistant**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Ampicillin	1	12.50	0	0.00	7	87.50
Ciprofloxacin	2	25.00	0	0.00	6	75.00
Cotrimoxazole	2	25.00	0	0.00	6	75.00
Ceftriaxone	6	75.00	1	12.50	1	12.50
Nalidixic acid	2	25.00	0	0.00	6	75.00
Nitrofurantoin	5	62.50	0	0.00	3	37.50
Norfloxacin	3	37.50	0	0.00	5	62.50
Amikacin	8	100	0	0.00	0	0.00

5.6.2 Antibiotic susceptibility pattern of the *Klebsiella pneumoniae* isolates

All the 3 *Klebsiella pneumoniae* isolates were sensitive to amikacin and ceftriaxone, 1(33.33%) intermediate to nitrofurantoin whereas all were resistant to ampicillin, ciprofloxacin, cotrimoxazole, nalidixic acid and norfloxacin (Table 7).

Table 7. Antibiotic susceptibility pattern of the *Klebsiella pneumoniae* isolates (n=3)

Antibiotics used	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Ampicillin	0	0.00	0	0.00	3	100
Ciprofloxacin	0	0.00	0	0.00	3	100
Cotrimoxazole	0	0.00	0	0.00	3	100
Ceftriaxone	3	100	0	0.00	0	0.00
Nalidixic acid	0	0.00	0	0.00	3	100
Nitrofurantoin	0	100	1	33.33	2	66.67
Norfloxacin	0	0.00	0	0.00	3	100
Amikacin	3	100	0	0.00	0	0.00

5.6.3 Antibiotic susceptibility pattern of the *Acinetobacter* spp. isolates

Both the isolates (100%) of *Acinetobacter* spp. were sensitive to amikacin whereas both were resistant to ampicillin, ciprofloxacin, cotrimoxazole, ceftriaxone, nitrofurantoin and norfloxacin (Table 8)

Table 8. Antibiotic susceptibility pattern of the *Acinetobacter* spp. isolates (n=2)

Antibiotics used	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Ampicillin	0	0.00	0	0.00	2	100
Ciprofloxacin	0	0.00	0	0.00	2	100
Cotrimoxazole	0	0.00	0	0.00	2	100
Ceftriaxone	0	0.00	0	0.00	2	100
Nitrofurantoin	0	0.00	0	0.00	2	100
Norfloxacin	0	0.00	0	0.00	2	100
Amikacin	2	100	0	0.00	0	0.00

5.6.4 Antibiotic susceptibility pattern of the *Pseudomonas aeruginosa* isolate

The isolated *Pseudomonas aeruginosa* was sensitive to amikacin whereas resistant to ciprofloxacin, cotrimoxazole, ceftriaxone, nitrofurantoin and norfloxacin (Table 9).

Table 9. Antibiotic susceptibility pattern of the *Pseudomonas aeruginosa* isolate (n=1)

Antibiotics used	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Ciprofloxacin	0	0.00	0	0.00	1	100
Cotrimoxazole	0	0.00	0	0.00	1	100
Ceftriaxone	0	0.00	0	0.00	1	100
Nitrofurantoin	0	0.00	0	0.00	1	100
Norfloxacin	0	0.00	0	0.00	1	100
Amikacin	1	100	0	0.00	0	0.00

5.6.5 Antibiotic susceptibility pattern of the *Staphylococcus aureus* isolates

Both the isolates (100%) of *Staphylococcus aureus* were sensitive to vancomycin whereas resistant to ampicillin, ciprofloxacin, cephalexin, cloxacillin and erythromycin. (Table 10)

Table 10. Antibiotic susceptibility pattern of the *Staphylococcus aureus* isolates (n=2)

Antibiotics used	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Ampicillin	0	0.00	0	0.00	2	100
Ciprofloxacin	0	0.00	0	0.00	2	100
Cephalexin	0	0.00	0	0.00	2	100
Cloxacillin	0	0.00	0	0.00	2	100
Erythromycin	0	0.00	0	0.00	2	100
Vancomycin	2	100	0	0.00	0	0.00

5.6.6 Antibiotic susceptibility pattern of the *Enterococcus* spp. isolates

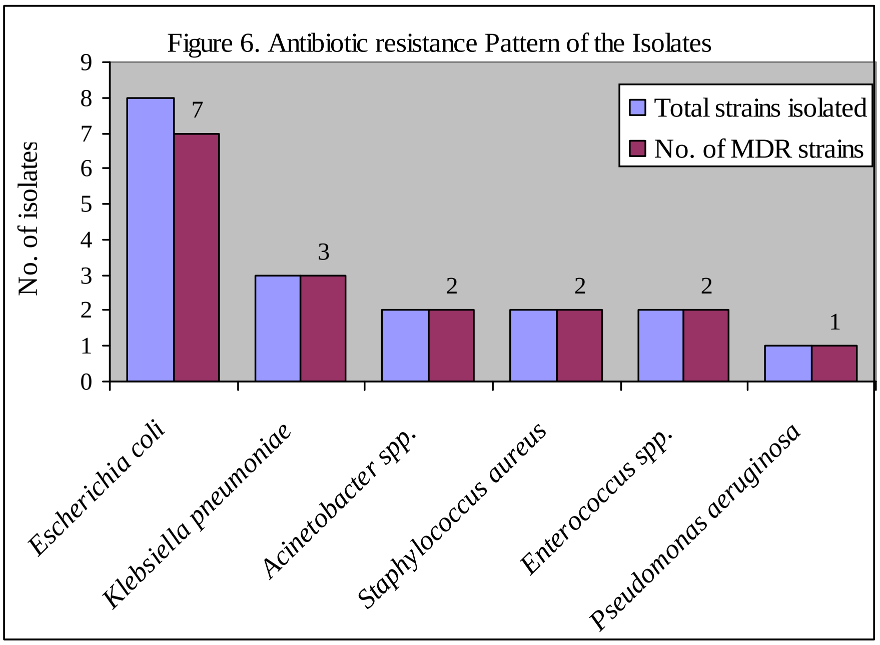
Both the isolates (100%) of *Enterococcus* spp. were sensitive to vancomycin and resistant to ampicillin, ciprofloxacin, cephalexin, cloxacillin and erythromycin (Table 11).

Table 11. Antibiotic susceptibility pattern of the *Enterococcus* spp. isolates (n=2)

Antibiotics used	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Ampicillin	0	0.00	0	0.00	2	100
Ciprofloxacin	0	0.00	0	0.00	2	100
Cephalexin	0	0.00	0	0.00	2	100
Cloxacillin	0	0.00	0	0.00	2	100
Erythromycin	0	0.00	0	0.00	2	100
Vancomycin	2	100	0	0.00	0	0.00

5.7 Antibiotic resistance pattern of the isolates

All the isolates i.e. 100% of *Klebsiella pneumoniae*, *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus and Enterococcus* spp. were found to be MDR strains whereas 87.50% of *Escherichia coli* were MDR strains (Figure 2)



CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

Infections are serious and often life threatening in cardiac surgery patients (Michalopoulos et al., 2006). Antibiotics used to treat life threatening diseases change the host's flora predisposing to colonization with multiple drug resistant bacteria, while others impair host defense mechanisms (Sharma, 2002). Adequate measures should, therefore, be implemented to diagnose and reduce the incidence of postoperative infections.

This study was conducted from March to September, 2008 to determine the prevalence of significant bacteriuria among postoperative patients who had undergone open heart surgery at Shahid Gangalal National Heart Centre, Bansbari.

One hundred and fifty one midstream urine samples from postoperative patients who had undergone open heart surgery were collected and the samples were processed for culture by semi quantitative standard loop method since urine culture is the gold standard for detection of asymptomatic bacteriuria (Leigh, 1990). The use of the clean-void midstream method of collection and semi quantitative urine cultures to differentiate infection from contamination is well established (Hooton et al., 2000). No currently available screening tests have a high enough sensitivity and negative predictive value for asymptomatic bacteriuria to replace the urine culture (Lin and Fajardo, 2008).

Out of 18 significant bacteriuric cases, 17 cases were asymptomatic whereas only one was symptomatic. Asymptomatic bacteriuria in this study was defined as the quantitative growth of bacteria, greater than or equal to 10^5 cfu/ml urine of the same organism, on aseptically collected midstream urine specimens, in the absence of symptoms of urinary tract infection (Cumming et al., 2006, Matteucci et al., 2007).

The prevalence of asymptomatic bacteriuria was 11.26% among postoperative patients who had undergone open heart surgery. This is comparable to other prevalence estimates of asymptomatic bacteriuria in postoperative patients undergoing arterial bypass surgery (Ploeg et al., 2007), hospitalized patients (Hyams, 1987), sickle cell patients (Cumming et al., 2006), school going children (Kumar et al., 2002) and patients with gallbladder disease (Vaishnavi et al., 2004) but higher than those reported in sexually active non-surgical healthy young women (Hooton et al., 2000).

Asymptomatic bacteriuria was higher among female patients than male but was not found to be statistically significant (P=0.22). The sexual dimorphism in the prevalence of asymptomatic bacteriuria has been reported in healthy adults. It is thought that this may be related to a relative deficiency of secretory IgA antibody in the urogenital tract of females compared with males (Cumming et al., 2006).

In this study, females of age group of 20-30 years were reported to have higher percentage of asymptomatic bacteriuria. This finding corresponds to the results of studies done by Hooton et al., 2000 that asymptomatic bacteriuria in young women is common and is a strong predictor of subsequent symptomatic urinary tract infection.

The highest percentage of significant bacteriuric cases were observed among valve surgery (15.91%) followed by vascular surgery (10.52 %). Compromised host resistance might be more significant in patients with valvular heart disease. Extended stay in hospitals appears to predispose the patients to infection with antibiotic resistant bacteria. This may be partly due to the greater likelihood of patients becoming colonized with such bacteria, from either horizontal nosocomial transmission or endogenous emergence of resistance, if the patient remains in the hospital for prolonged duration (Pawar et al., 2008).

Out of 18 cases with significant bacteriuria, 77.78 % were due to gram negative rods and 22.22% were due to gram positive cocci. Among the total isolates, *E. coli* (44.44%) was found to be predominant followed by *Klebsiella pneumoniae*. The other bacterial isolates were *Acinetobacter* spp., *Staphylococcus aureus* and *Enterococcus* spp.; all the three accounting for 11.11% of the total isolates and *Pseudomonas aeruginosa* constituted 5.56%.

Higher prevalence of *E. coli* seen in this study resembled the study done by various other workers viz.; Badami and Deodhar, 1976; Boyko et al., 2005; Cumming et al., 2006; Hermida and Ferrer, 2004; Jayalakshmi, 2008; Jha and Singh, 2007; Keah et al., 2007; Kumar et al., 2002; Lin et al., 2006; Matteucci et al., 2007; Naylon, 1984; Turan et al., 2008 and Ullah et al., 2007. The ability of UPEC (Uropathogenic *E. coli*) strains to colonize the human urinary tract and cause symptomatic UTI is facilitated by a number of virulence factors such as fimbriae (P and type 1), K antigens, siderophores, haemolysins, serum resistance, urease production, motility, etc. On the other hand, ABU (Asymptomatic bacteriuric) *E. coli* strain manages to colonize the human urinary tract by two primary strategies viz.; fast growth and biofilm formation (Klemm et al., 2007).

Klebsiella pneumoniae was the second principal isolate among gram negative bacilli constituting 16.67% of the total isolates. Similar type of result was reported by Turan et al., 2008 and Badami and Deodhar, 1976. In a study carried out by Jha and Singh, 2007 in the Pokhara valley, this species represented 14.28% of the total isolates.

Acinetobacter spp. constituted 11.11% of the total isolates in this study. *Acinetobacte*r spp has recently emerged as a major cause of hospital acquired infections because of the extent of its antimicrobial resistance and its propensity to cause large, often multi-facility nosocomial outbreaks. The combination of high innate antibiotic resistance and selective antibiotic pressure has resulted in the emergence of *Acinetobacte*r spp. as important nosocomial pathogen (Takahashi et al., 2000).

Pseudomonas aeruginosa was isolated in only one bacteriuric case in this study. This bacterium is considered as a primary pathogen in compromised hosts and hospitalized patients (Dolan et al., 1989). *P. aeruginosa* is the leading cause of nosocomial infections which rank second among the gram negative pathogens reported to the National Nosocomial Infection Surveillance System (Carmel et al., 1999).

Two different species of gram positive cocci were isolated; *Staphylococcus aureus* and *Enterococcus* spp., both constituting 11.11% of the total isolates. This is in agreement with that reported by Jha and Singh, 2007. A pure culture of *S. aureus* is considered to be significant regardless of the number of cfus and antimicrobial susceptibility test should be performed (Forbes et al., 2007).

Antimicrobial resistance is an issue of great significance for public health at the global level. The increasing antibiotic resistance problems, largely due to widespread and irrational use of antimicrobial agents in hospitals and the community, is a cause of great concern, especially in developing countries. Hospital antibiograms can be a useful means for guiding empiric therapy and tracking the emergence of bacterial resistance (Lakshmi, 2008). Therefore, the isolates from significant bacteriuric cases were subjected to in vitro antibiotic susceptibility testing by Kirby Bauer disc diffusion method.

All the gram negative isolates were found to be sensitive to amikacin. In a similar study done by Chandrashekhar et al., 2006, amikacin was highly sensitive towards gram negative organisms. Pawar et al., 2008 also reported 100% susceptibility of *Acinetobacter* spp. to amikacin. In our study, 64.28% of the gram negative isolates were sensitive to ceftriaxone followed by nitrofurantoin with a susceptibility of 35.71% whereas most of them i.e. 92.31% were resistant to ampicillin. Among the *E. coli* isolates, 75%were found to be resistant to nalidixic acid. Eom et al., 2002 reported that incidence of quinolone resistant *E. coli* (QREC) increased from 14.4 to 21.3% during 5 years from 1996 to 2000 in Korea.

Among the four gram positive isolates, all were sensitive to vancomycin. This is in agreement with the finding of Niederhäuser et al., 1997 who reported 100% sensitivity of *Staphylococcus aureus* and CONS to vancomycin. All the gram positive isolates were resistant to ampicillin, ciprofloxacin, cloxacillin, cephalexin and erythromycin.

All the isolates of *Klebsiella pneumoniae*, *Acinetobacter* spp., *Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus* spp. were found to be MDR strains whereas 87.51% of *Escherichia coli* were MDR strains. The defining criterion for multidrug resistance (MDR) in this study was resistance to ≥ 2 of the antimicrobial agents belonging to different structural classes (Dahal et al., 2005).

This high resistance of bacterial isolates to the commonly used antibiotics might be due to the fact that the urine samples were collected from cardiac patients who underwent open heart surgery and were under routine postoperative administration of prophylactic antibiotics.

Antimicrobial prophylaxis is indicated for any clean operation that involves the use of prosthetic materials (e.g. joint replacements, vascular and cardiac surgery), wherein the consequences of surgical-site infections would be devastating. (Meneshian and Meakins, 2005). Nicolle et al., 1987 reported that antimicrobial therapy was associated with an increased incidence of reinfection and adverse antimicrobial drug effects as well as isolation of increasingly resistant organisms in recurrent infection when compared with no therapy in institutionalized elderly women with asymptomatic bacteriuria. The abundant use of antimicrobial drugs particularly in hospitalized patients leads to the suppression of drug susceptible organisms in the gut flora and favours the persistence and growth of drug resistant bacteria including *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp. and *Serratia* spp. (Brooks et al., 2001). Wettergren and Jodal, 1990 mentioned that antibiotic treatment can decrease the host colonization resistance by disturbing the ecological balance in the periurethral region.

All the urine samples were collected from patients at least 48 hours after removal of urinary catheter. Patients in this study were under urinary catheterization during and after operation for a short period of time depending upon the condition of the patients. When a urinary catheter is exposed to urine, various components adsorb onto the surface and form a conditioning film, which becomes the real interface where microbial interaction takes place. When a biofilm has established in a catheter, the inherent tolerance/resistance of biofilm-dwelling bacteria towards antibiotics often make such infections extremely recalcitrant and hard to eradicate (Ferrie` res et al., 2007).

Since the patients in this study were under routine postoperative administration of prophylactic antibiotics, this might have suppressed endogenous microflora thereby permitting the overgrowth of pathogenic strains, development or selection of antimicrobial agent resistant strains and disruption of colonization resistance.

The principal limitations of this study lie in the fact that follow up of the asymptomatic bacteriuric patients for any symptomatic urinary tract infections was not done. The high resistance of bacterial isolates to the commonly used antibiotics suggest further systematic study be done extensively in this area.

6.2 CONCLUSION

A significant number of urinary isolates from postoperative cardiac patients were MDR which can result in unavoidable treatment failure. Therefore, the rationale use of antibiotics is suggested.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

1. Out of 151 mid stream urine samples processed for culture by semiquantitative standard loop method, 18 (11.92%) samples showed significant bacteriuria, 35 (23.17%) samples showed insignificant growth while 98 (64.90%) samples showed no growth.

2. Among 18 significant bacteriuric cases, 17 were asymptomatic while 1 case was symptomatic.

3. The prevalence of asymptomatic bacteriuria was found to be 11.26% among the postoperative patients who had undergone open heart surgery

4. Significant bacteriuria was found to be higher in females 61.11% than males but was not found to be statistically significant ($P>0.05$).

5. The highest percentage of significant bacteriuric cases was observed among valve surgery (15.91%) followed by vascular surgery (10.52 %).

6. The predominant bacteria among the bacteriuric strains were the gram negative rods constituting 77.78% and gram positive bacteria constituted only 22.22% of the total isolates.

7. Altogether six different species of bacteria were isolated among which *Escherichia coli* (44.44%) was found to be the predominant followed by *Klebsiella pneumoniae* (16.67%). The other organisms were *Acinetobacter* spp. (11.11%),

Staphylococcus aureus (11.11%), *Enterococcus* spp. (11.11%) and *Pseudomonas aeruginosa* (5.56%).

8. All the gram negative isolates were sensitive to amikacin followed by ceftriaxone with a susceptibility of 64.28% and nitrofurantoin with a susceptibility of 35.71%.Most of the gram negative isolates i.e. 92.31% were resistant to ampicillin.

9. All the gram positive isolates were sensitive to vancomycin whereas all of them were resistant to ampicillin, ciprofloxacin, cloxacillin, cephalexin and erythromycin.

10. All the isolates i.e. 100% of *Klebsiella pneumoniae*, *Acinetobacter* spp., *Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus* spp. were found to be MDR strains whereas 87.51% of *Escherichia coli* were MDR strains.

7.2 RECOMMENDATIONS

1. Since 87.50% of *Escherichia coli* isolates were found to be MDR strains, standard laboratory procedures should be used to detect resistance as an aid to managing the infected patient and as a means of monitoring changing resistance trends among clinically relevant bacteria.

2. Hospital environment should be monitored routinely so as to trace the source of infection and hence preventive measures could be employed in time.

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APPENDIX-I

QUESTIONNAIRE

CLINICAL AND MICROBIOLOGICAL PROFILE OF PATIENT

Clinical Profile

Date

Name: … … … … … … … … … … … … … …   IP No. :… …

Age / Sex: … … … … … … … …… … … … …   Ward / Bed No:….

Address: … … … … … … … … … … … … … …

Brief Clinical History

Date of admission:………………..

Date of operation:………………

Patient on antibiotics   Yes   No

If Yes, Antibiotic(s) taken: 1) … … … … … … … …   2) … … … …… …

Duration of treatment: … … … …… … … …… … … …

Microbiological Profile

Day 1 ***(… … / … … / … … … …)***

Specimen: … … … … … … … …

Time of sample collection: … … … … … … … …

Mode of Collection: … … … … … … … …

Receiving time at the laboratory: … … … … … … … …

Inoculation on 1………………………..   2………………………..

Incubation temperature……………...   Incubation time………………

Day 2(--------/----/----)

Observation of culture plates: No Growth / Growth

Number of colonies:

Colony Characteristics:

Media used	Feature	Shape	Size	Colour	Texture	Opacity	Consistency

Gram staining results:

Catalase test: Oxidase test:

Coagulase test:

Provisional identification of the organism:

Inoculation on: 1………………… 2…………………

Day 3(--------/----/----)
Biochemical tests:

SIM ……………………. TSI ………………………….

Citrate ……………………. Urease …………………………….

Others ………………….

Organism identified as:

Antibiotic susceptibility testing by Kirby Bauer disc diffusion method

Antibiotics used	Conc. of antibiotic	Zone of Inhibition (mm)	Interpretation

APPENDIX-II

I.COMPOSITION AND PREPARATION OF DIFFERENT CULTURE MEDIA

The culture media used were from Hi-Media Laboratories Pvt. Limited, Bombay, India. (All compositions are given in grams per liter and at 25^{0}C temperature)

1. Blood agar (BA)

Blood agar base (infusion agar) + 5-10% sheep blood

Ingredients	**gm/liter**
Beef heart infusion	500.0
Tryptose	10.0
Sodium Chloride	5.0
Agar	15.0

Final pH (at 25^{0}C) 7.3±0.2

Blood agar base medium (42.5 grams) was suspended in 1000 ml distilled water and sterilized by autoclaving at 121^{0}C (15lbs pressure) for 15 minutes. After cooling to 40-50^{0}C, 50 ml sterile defibrinated sheep blood was added aseptically and mixed well before pouring.

2. MacConkey Agar (MA)

Ingredients	**gm/liter**
Peptone	20.0
Lactose	10.0
Sodium taurocholate	5.0
Sodium chloride	5.0
Neutral Red	0.04
Agar	20.0

Final pH (at 25^{0}C) 7.4±0.2

MacConkey Agar medium (55 grams) was suspended in 1000 ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121^0C (15 lbs pressure) for 15 minutes.

3. **Mueller Hinton Agar (MHA)**

Ingredients	**gm/liter**
Beef, Infusion form	300.0
Casein Acid Hyrolysate	17.5
Starch	1.5
Agar	17.0

Final pH (at 25^0C) 7.4±0.2

The medium (38 grams) was suspended in 1000 ml distilled water and the medium was warmed to dissolve. 10 ml was distributed in test tubes and sterilized by boiling in water bath for 10 minutes.

4. **Nutrient Agar (NA)**

Ingredients	**gm/litre**
Peptone	10.0
Sodium Chloride	5
Beef Extract	10.0
Yeast Extract	1.5
Agar	12.0

Final pH (at 25^0C) 7.4±0.2

The medium (37 grams) was suspended in 1000 ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121^0C (15 lbs pressure) for 15 minutes.

5. **Nutrient Broth (NB)**

Ingredients	**gm/litre**
Peptone	5.0

Sodium Chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5

Final pH (at 25^0C) 7.4±0.2

The medium (13 grams) was dissolved in 1000 ml distilled water and autoclaved at 121˚C for 15 minutes.

II. Biochemical Test Media

1. Sulphide Indole Motility (SIM) medium

Ingredients	**gm/litre**
Beef Extract	3.0
Peptone	30.0
Peptonized Iron	0.2
Sodium Thiosulphate	0.025
Agar	3.0

Final pH (at 25^0C) 7.3±0.2

The medium (36 grams) was suspended in 1000 ml distilled water and dissolved completely. Then it was distributed in tubes to a depth of about 3 inches and sterilized.

2. Simmon's Citrate Agar

Ingredients	**gm/litre**
Magnesium Sulfate	0.2
Mono-ammonium Phosphate	1.0
Dipotassium Phosphate	1.0
Sodium Citrate	2.0
Sodium Chloride	5.0
Agar	15.0
Bromothymol Blue	0.08

Final pH (at 25^0C) 6.8±0.2

The medium (24.2 grams) was dissolved in 1000ml distilled water. 3ml medium was distributed in test tubes and sterilized by autoclaving at 121^0C for 15 minutes. After autoclaving tubes containing medium were tilted to form slant.

3. Triple Sugar Iron (TSI) Agar

Ingredients	gm/litre
Peptone	10.0
Tryptone	10.0
Yeast Extract	3.0
Beef Extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous Sulphate	0.2
Sodium Chloride	5.0
Sodium Thiosulphate	0.3
Phenol Red	0.024
Agar	12.0

Final pH (at 25^0C) 7.4±0.2

The medium (65 grams) was dissolved in 1000ml of distilled water and sterilized by autoclaving at 15 lbs (121^0C) pressure for 15 minutes. The medium was allowed to set in sloped form with a butt about 1 inch of thickness.

4. Christensen Urea Agar

Ingredients	gm/litre
Peptone	1.0
Dextrose	1.0
Sodium Chloride	5.0
Dipotassium Phosphate	1.2
Mono-potassium Phosphate	0.8
Phenol Red	0.012
Agar	15.0

Final pH (at 25^0C) 7.4±0.2

The medium (24 grams) was suspended in 950 ml distilled water and sterilized by autoclaving at121^0C for 15 minutes. After cooling to about 45^0C, 50 ml of 40% urea was added and mixed well. Then 5 ml was dispensed in test tube and set at slant position.

III. Staining and Test Reagents

1. For Gram's Stain

(a) Crystal Violet solution

Crystal Violet	20.0 g
Ammonium Oxalate	9.0 g
Ethanol or Methanol	95 ml

Distilled Water (D/W) to make 1 litre

Preparation: In a clean piece of paper, 20 gm of crystal violet was weighed and transferred to a clean brown bottle. Then, 95 ml of ethanol was added and mixed until the dye was completely dissolved. To the mixture, 9 gm of ammonium oxalate dissolved in 200 ml of D/W was added. Finally the volume was made 1 litre by adding D/W.

(b) Lugol's Iodine

Potassium Iodide	20.0 g
Iodine	10.0 g
Distilled Water	1000 ml

Preparation: To 250 ml of D/W, 20 gm of potassium iodide was dissolved. Then 10 gm of iodine was mixed to it until it was dissolved completely. Finally the volume was made 1 litre by adding D/W.

(c) Acetone-Alcohol Decoloriser

Acetone	500 ml
Ethanol (Absolute)	475 ml
Distilled Water	25 ml

Preparation: To 25 ml D/W, 475 ml of absolute alcohol was added, mixed and transferred into a clean bottle. Then immediately, 500 ml acetone was added to the bottle and mixed well.

(d) Safranin (Counter Stain)

Safranin	10.0 g
Distilled Water	1000 ml

Preparation: In a clean piece of paper, 10 gm of safranin was weighed and transferred to a clean bottle. Then 1 litre D/W was added to the bottle and mixed well until safranin dissolved completely.

2. Normal saline

Sodium Chloride	0.85 g
Distilled Water	100 ml

Preparation: The sodium chloride was weighed and transferred to a leak-proof bottle premarked to hold 100 ml. Distilled water was added to the 100 ml mark, and mixed until the salt was fully dissolved. The bottle was labeled and stored at room temperature.

TEST REAGENTS

a. For Catalase test

Catalase Reagent (3% H_2O_2)

Hydrogen peroxide	3 ml
Distilled Water	97 ml

Preparation: To 97 ml of D/W, 3 ml of hydrogen peroxide was added and mixed well.

b. For Oxidase Test

Oxidase Reagent (impregnated in Whatman's No. 1 filter paper)

Tetramethyl *p*-phenylene diamine dihydrochloride (TPD)	1 gm
Distilled Water	100 ml

Preparation: This reagent solution was made by dissolving 1 gm of TPD in 100 ml D/W. To that solution strips of Whatman's No. 1 filter paper were soaked and drained for about 30 seconds. Then these strips were freeze dried and stored in a dark bottle tightly sealed with a screw cap.

c. For Indole Test

Kovac's Indole Reagent

Isoamyl alcohol	30 ml
p-dimethyl aminobenzaldehyde	2.0 g
Hydrochloric acid	10 ml

Preparation: In 30 ml of isoamylalcohol, 2 g of *p*-dimethyl aminobenzaldehyde was dissolved and transferred to a clean brown bottle. Then to that, 10 ml of conc. HCl was added and mixed well.

d. Mac Farland standard 0.5

It is prepared by adding 0.6 ml of 1% w/v barium chloride solution to 99.4 ml of 1% v/v solution of sulphuric acid.

APPENDIX-III

A. Gram-staining Procedure

1. A thin film of the material to be examined was prepared and dried.
2. The material on the slide was heat fixed and allowed to cool before staining.
3. The slide was flooded with crystal violet stain and allowed to remain without drying for 10-30 seconds.
4. The slide was rinsed with tap water, shaking off excess.
5. The slide was flooded with iodine solution and allowed to remain on the surface without drying for twice as long as the crystal violet was in contact with the slide surface.
6. The slide was rinsed with tap water, shaking off excess.

7. The slide was flooded with alcohol acetone decolorizer for 10 seconds and rinsed immediately with tap water until no further color flows from the slide with the decolorizer. Thicker smear requires more aggressive decolorizing.
8. The slide was flooded with counter stain (safranin) for 30 seconds and washed off with tap water.
9. The slide was blotted between two clean sheets of bibulous paper and examined microscopically under oil immersion at 1000X.

B. Standardization of Loop

The wire loop was calibrated by the following procedures

1. A small container (e.g., bijou bottle of distilled water) was weighed.
2. Using a loop, 100 loopfuls of water was removed onto a blotting paper.
3. The container and water was reweighed.
4. The difference in weight was calculated.
5. The volume held by the loop was the difference in weight divided by 100.
6. The procedure was performed three times and the average of the three results was taken.

APPENDIX-IV

LIST OF EQUIPMENTS AND MATERIALS USED DURING THE STUDY

1. Equipments:

Oven Ambassadors, Laboratory Electronics Oven
Incubator Micro processor temperature control;Australia;Clayson IM1000
Autoclave Sakura Tiyoda Manufacturing Company, Japan

Refrigerator LG, Express cool
Microscope Olympus, PM-10 ADS: Olympus Optical Co. Ltd, Japan
Centrifuge Kubota Model KC 25, Japan
Weighing Machine SC 2020/3EO, SCO4T11 200g*0.1g Ohaus Corporation, Florham Park, NJ 07932, USA
Water Distillation Plant Advantec. GS-200, Japan

1. **Microbiological media (Hi-Media)**

Mac Conkey Agar
Blood Agar
Agar
Nutrient broth
Mannitol salt Agar
Mueller-Hinton Agar
TSI Agar
Simmons Citrate
Urease Agar
SIM

2. **Chemicals/ Reagents**

3% hydrogen peroxide
Acetone-alcohol
Safranine
Normal Saline
tetramethyl paraphenyl diamine dihydrochloride
Crystal violet
Gram's iodine
Kovac's reagent

3. **Antibiotics Discs (Hi-Media)**

Amikacin
Ampicillin
Ceftriaxone
Cephalexin
Ciprofloxacin
Vancomycin
Cloxacillin
Cotrimoxazole
Erythromycin
Nitrofurantoin
Norfloxacin

4. **Miscellaneous**

Glasswares
Forceps, droppers
cotton Tissue paper
Distilled water
Lysol
Inoculating loop,
Blotting paper,
Immersion oil
Sticker

ZONE SIZE INTERPRETATIVE CHART

Antimicrobial Agents used	Symbol	Disc Content	Resistant	Intermediate	Sensitive (mm

			(mm or less)	(mm)	or more)
Ampicillin When testing gram negative enteric organism When testing staphylococci	A	10µg	13 28	14-16 –	17 29
Ceftriaxone	Ci	30 µg	13	14-20	21
Cotrimoxazole	Co	1.25/23.75µg	10	11-15	16
Cloxacillin	Cx	1 µg	12	12-13	14
Erythromycin	E	15 µg	13	14-22	23
Ciprofloxacin	Cf	5 µg	15	16-20	21
Norfloxacin	Nx	10 µg	12	13-16	17
Nalidixic Acid	Na	30µg	13	14-18	19
Nitrofurantoin	Nf	300µg	14	15-16	17

(Source: Product Information Guide, Hi-Media Laboratories Pvt. Limited, Bombay, India).

APPENDIX-V

Table: Distinguishing reactions of the commoner and pathogenic Enterobacteriaceae

Species	Test/ substrate											
	lac	Mot	gas	ind	VP	cit	PDA	ure	lys	H_2S	inos	ONPG
E. coli	+	+	+	+	-	-	-	-	+	-	-	+
Shigella groups A, B, C	-	-	-	±	-	-	-	-	-	-	-	-
Sh. sonnei	-	-	-	-	-	-	-	-	-	-	-	+
Salmonella (most serotypes)	-	+	+	-	-	+	-	-	+	+	±	-
S. typhi	-	+	-	-	-	-	-	-	+	+	-	-
S. paratyphi A	-	+	+	-	-	-	-	-	-	-	-	-
C. freundii	±	+	+	-	-	+	-	±	-	±	-	+
C. koseri	±	+	+	+	-	+	-	±	-	-	-	+
K. pneumoniae	+	-	++	-	+	+	-	+	+	-	+	+
K. oxytoca	+	-	++	+	+	+	-	+	+	-	+	+
E. aerogenes	+	+	++	-	+	+	-	-	+	-	+	+
E. cloacae	+	+	+	-	+	+	-	±	-	-	-	+
Hafnia alvei	-	+	+	-	+	-	-	-	+	-	-	+
Serratia marcescens[b]	-	+	±	-	+	+	-	-	+	-	±	+
P.mirabilis	-	+	+	-	±	±	+	++	-	+	-	-
P. vulgaris	-	+	+	+	-	-	+	++	-	+	-	-
M. morganii	-	+	+	+	-	-	+	++	-	±	-	-
Providencia rettgeri	-	+	-	+	-	+	+	++	-	-	+	-
P. stuartii	-	+	-	+	-	+	+	±	-	-	+	-
P. alcalifaciens	-	+	+	+	-	+	+	-	-	-	-	-
Yersinia enterocolitica[c]	-	-	-	±	-	-	-	±	-	-	±	+
Y. pestis	-	-	-	-	-	-	-	-	-	-	-	±
Y. pseudotuberculosis	-	-	-	-	-	-	-	+	-	-	-	±

[a] lac, inos, fermentation of lactose, inositol; mot, motility; gas, gas from glucose; ind, indole production; VP, Voges-Proskauer; cit, Citrate utilization (Simmons'); PDA, phenylalanine deaminase; ure, urease; lys, lysine decarboxylase; H_2S, H_2S produced in TSI agar; ONPG, metabolism of *o*-nitrophenyl-β-D-galactopyranoside.
[b] Some strains of Serratia marcescens may produce a red pigment
[c] *Yersinia* are motile at 22°C.

{Key: +, ≥85% of strains positive; -, ≥ 85% of strains negative; 16-84% of strains are positive after 24-48 hour at 36°C}
(Source: Collee *et al*, 1996)

APPENDIX-VI

ASSOCIATION OF SIGNIFICANT BACTERIURIA WITH GENDER

Gender	Significant bacteriuria	Insignificant bacteriuria	P- Value*
Male	7	74	**0.222**
Female	11	59	

***** calculated using Pearson's $\Re^2$

(Statistical analysis was done using SPSS version 14.0)