MICROBIOLOGICAL INVESTIGATION AND DISINFECTION POLICY IN AN I.C.U

Thesis
Submitted for Partial Fulfillment of M.D. Degree
In Clinical and Chemical Pathology

By
Safaab Ibrahim Abbas Mansour
M.SC. Degree (Benha)
In clinical and Chemical Pathology

Supervisors
PROF. DR. FETNAT MAHMOUD TOLBA
Prof. of Clinical and Chemical Pathology
Benha Faculty of Medicine
Zagazig University

PROF. DR. SOHEIR ABD EL RAHMAN ABD EL SAMEA
Prof. of Clinical and Chemical Pathology
Benha Faculty of Medicine
Zagazig University

DR. ANAS ABD EL RAHMAN YOUSEF
Ass. Prof. of Clinical and Chemical Pathology
Benha Faculty of Medicine
Zagazig University

DR. NAGWA ABD EL GHANY KHAMIS
Assistant Consultant of Clinical and Chemical Pathology
Ain Shams University
Specialized Hospital

Benha Faculty of Medicine
Zagazig University

2004
بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

وَأَنْقِرُوا اللَّهِ وَبَيْنَكُمْ اللَّهُ

وَاللَّهُ يَكُونَ عَلَيْهِ شَفَاءً

صَرَّحُ اللَّهُ العَظِيمُ

سُورَةُ البقرة آية٢٨٢
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<td>Aeromonas hydrophila</td>
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<td>Amp. C</td>
<td>Ampicillinases</td>
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<td>AgSD</td>
<td>Silver sulfadiazine</td>
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<td>B. subtilis</td>
<td>Bacillus subtilis</td>
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<td>BEAA</td>
<td>Bile esculin azide agar</td>
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<td>BHI</td>
<td>Brain heart infusion</td>
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<td>C. Albicans</td>
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<td>C. krusei</td>
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<td>C.I.T</td>
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<td>C. lusitaniae</td>
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<td>C. freundii</td>
<td>Citrobacter freundii</td>
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<tr>
<td>C. koseri</td>
<td>Citrobacter koseri</td>
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<tr>
<td>C. Difficile</td>
<td>Clostridium difficile</td>
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<tr>
<td>CDC</td>
<td>Centres for disease control and prevention</td>
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<td>CFU</td>
<td>Colony forming unit</td>
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<td>CNS</td>
<td>Coagulase negative staph.</td>
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<td>CRA₅</td>
<td>Chlorine – releasing agents</td>
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<td>EIEC</td>
<td>Enteroinvasive escherichia coli</td>
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<td>EPIC</td>
<td>European prevalence of infection in intensive care</td>
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<td>ESβL₅</td>
<td>Extended spectrum β Lactamases</td>
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<td>ETEC</td>
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<td>ETO</td>
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<td>GEL</td>
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<td>GIT</td>
<td>Gastro intestinal tract</td>
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<td>GPS</td>
<td>Gram positive susceptibility system</td>
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<td>H₂O₂</td>
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<td>HAI</td>
<td>Hospital acquired infection</td>
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<td>HAUTI</td>
<td>Hospital acquired urinary tract infection</td>
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<td>H₂S</td>
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<td>I.V.</td>
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<td>K. pneumoniae</td>
<td>Klebsiella pneumoniae</td>
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<tr>
<td>LDC</td>
<td>Lysine decarboxylase</td>
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<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>MBC</td>
<td>Minimal bactericidal concentration</td>
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<td>MCCU</td>
<td>Medical critical care unit</td>
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<td>MDR – T.B</td>
<td>Multidrug resistant – tuberculosis</td>
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<td>MHA</td>
<td>Muller hinton agar</td>
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<tr>
<td>MHB</td>
<td>Muller hinton broth</td>
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<tr>
<td>MIC</td>
<td>Minimal inhibitory concentration</td>
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<td>MRSA</td>
<td>Methicillin resistant S.aureus</td>
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<td>MRSE</td>
<td>Methicillin resistant S. epidermidis</td>
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<td>NCCLS</td>
<td>National committee for clinical laboratory standards</td>
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<td>NIT</td>
<td>Nitrate reduction</td>
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<td>ODC</td>
<td>Ornithine</td>
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<td>PAL</td>
<td>Alkaline phosphatase</td>
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<td>Proteus mirabilis</td>
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<td>Pseud. aeruginosa</td>
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<td>PAA</td>
<td>Peracetic acid</td>
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<td>PBP</td>
<td>Penicillin binding protein</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PFGE</td>
<td>Pulsed field gel electrophoresis</td>
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<td>PHMB</td>
<td>Polyhexamethylene biguanides</td>
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<td>REA</td>
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<td>RFLP</td>
<td>Restriction fragment length polymorphism</td>
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<td>RNA</td>
<td>Ribonucleic acid</td>
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<td>RS - PCR</td>
<td>Ribosome spacer polymerase chain reaction</td>
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<td>Staphylococcus epidermidis</td>
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<td>S. pyogenes</td>
<td>Streptococcus pyogenes</td>
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<td>S. saprophyticus</td>
<td>Staphylococcus saprophyticus</td>
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<td>SASPs</td>
<td>Small acid-soluble spore protein</td>
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<td>SCCU</td>
<td>Surgical critical care unit</td>
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<td>TCC</td>
<td>Triclorocarbon</td>
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<td>TDEs</td>
<td>Transmissible degenerative encephalopathies</td>
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<td>TSA</td>
<td>Triple sugar agar</td>
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<tr>
<td>TSB</td>
<td>Triple sugar broth, trypticase soy broth</td>
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<td>URE</td>
<td>Urease</td>
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<td>UTI</td>
<td>Urinary tract infection</td>
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<td>VAN-A, VAN-B, VAN-C</td>
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<td>VHP</td>
<td>Vaporized hydrogen peroxide</td>
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<td>VP</td>
<td>Voges-proskauer</td>
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<td>VRE</td>
<td>Vancomycin resistant enterococci</td>
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INTRODUCTION
INTRODUCTION

Antiseptics and disinfectants are extensively used in hospitals and other health settings for a variety of topical and hard surface application (McDonell and Russell, 1999). They are an essential part for infection control practices and aid in the prevention of nosocomial infections (Rutala, 1995).

Because it is unnecessary to sterilize all patient care items, hospital policies must identify whether cleaning, disinfection or sterilization is indicated, based primarily on the item’s intended use (Rutala, 1998).

Instruments and items for patient care were divided into three categories; critical, semi-critical, and non critical; the disinfection is the selective elimination of certain undesirable microorganisms (Reber et al., 1972).

The advantages and limitations of various disinfectants must be well known and appreciated. The disinfectant chosen must be not expensive, not toxic, not affected by environmental factor, surface compatibility, residual effect on treated surface, easy to use, odorless, soluble in water, stable, and should have good cleaning properties (Russell et al., 1983).

Nosocomial infection may be either endogenous or exogenous; endogenous infection caused by organisms that are present as part of normal flora of the patient, while exogenous infections are those caused by organisms acquired by exposure to hospital personnel, medical devices or hospital environment (Stamm, 1978).
The most common pathogen in hospital acquired infection are E. Coli, S. aureus, Enterococci and Pseudomonas aeruginosa (Horan et al., 1986).

Nosocomial infection in intensive care unit (ICU) is a frequent event with potentially lethal consequences. Because patients in intensive care are severely ill and undergo invasive procedures, they develop nosocomial infection more frequently than other hospitalized patients (Craven et al., 1988).

Common sites of nosocomial infection in an ICU differ from those sites in the ward, the three most common sites are the respiratory tract, the urinary tract, and the blood stream (Nystrom et al., 1988).

Studies have shown that during the first few hours to days of hospitalization, the patient's flora begins to change and becomes colonized with organisms that are present in the hospital environment (Holey, 1986).

Escherichia coli is the most common hospital acquired organisms and S. aureus is the second most common serious isolate from blood cultures (Anonymous, 1997).

It is now recognized that many Gram negative nosocomial pathogens have developed broad spectrum resistant to a newer beta lactam antibiotics through small modification in the structure of the enzymes that they are already posses. The extended spectrum beta-lactamases (ESBL) are particularly common in isolates of Escherichia
coli, Klebsiella, and Citrobacter they located on transmissible plasmids (Speller and Humphreys, 1998).

An important role of the microbiology laboratories is accurate and timely detection of multi-resistant strains which are the first line of defense (Willey et al., 1992).
AIM OF THE WORK
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The aim of this work is:

1) Study of the potential sources of nosocomial infections in an intensive care unit either by
   a) Isolating microorganisms from different environmental sites as air and equipments.
   b) Isolating potentially hazardous microorganisms from nose and hands of working staff.

2) Study of the effect of available disinfectants and antisepsics on the isolated microorganisms (from environment and working staff).

3) Screening of ICU patients for the presence of multi-drug resistant bacteria for the purpose of application of appropriate isolation measures.

By these surveillance studies, we can put a strategy for infection control in the ICU of Benha university hospital.
Review
NOSOCOMIAL INFECTION

A nosocomial infection or hospital acquired infection (HAI) is any infection developed during hospitalization, not present nor incubating at the time of hospital admission and is usually not manifested in the first 48 hours of hospitalization, though it may appear within 48 hours after discharge (Department of Health, 1995).

Nosocomial infections represent a major hazard in health care facilities, their effects are felt by the infected patients, their families and the health care system (Whitehouse et al., 1998).

The presence of nosocomial infection and the number of infection episodes were the variables with the strongest association with prolonged hospital stay among intensive care unit patients (Olaechea et al., 2003).

Incidence and frequency of nosocomial infection:

At least 5% of patients entering USA hospitals develop a nosocomial infection. In Great Britain and Ireland a prevalence rate of 9% has been reported. In Spain the prevalence rate was 7.2% (Speller and Humphreys, 1998).

The acquisition of nosocomial pathogens depends on a complex interplay of the host, pathogen and environment (Weber and Rutala, 1997).

In the 1950s and 60s Staphylococcus aureus (S. aureus) was the most important hospital pathogen. However gram-negative bacilli have become a major cause of nosocomial infections, and that critically ill patients admitted to an intensive care unit (ICU), rapidly change their
normal bacterial flora and acquire gram-negative hospital pathogens (Noone et al., 1983).

However, a study in Benha university hospital revealed that gram negative bacilli represented 73.45% of isolated microorganisms, klebsiella was the commonest microorganism isolated followed by E. coli, Pseudomonas aeruginosa, and Proteus mirabilis, while S. aureus was the commonest gram positive cocci isolated (Soheir, 1991).

However, gram positive cocci and candida species have replaced gram negative bacilli as the most common causes of nosocomial infections (Kloos & Bannerman, 1994; Pfaller, 1996). For example, the frequency of infection caused by E-coli has declined and the frequency of infections caused by Staphylococci, Enterococci and Candida has increased (Jones et al., 1994).

The most alarming recent trend are the increase frequency of multiresistant microorganisms for causing hospital acquired infection (HAI) (Breiman et al., 1994).

Types of nosocomial infection:

Body site distribution was investigated, with five sites predominating; urinary tract infection (commonest), then lower respiratory tract infection, surgical wound infection, blood stream infection and skin infection (Willson, 1995).

However, a study in Assuit university hospital reported that surgical wound infection is the commonest nosocomial infection in Egypt
(54%), then urinary tract infection and lower respiratory tract infection
(Ahmed et al., 1993).

However, a study in Benha university hospital found, the frequency of nosocomial infections were as follow urinary tract infections, wound infections, blood stream infection and lastly respiratory tract infection
(Soheir et al., 2002).

Kim et al., (2000) reported that the order was as follow, urinary tract infections, pneumonias, surgical site infections and blood stream infections.

Such difference may be due to specific hospital manipulation and general hygiene (Ahmed et al., 1993).

Classification of nosocomial infection:

Nosocomial infections have been classified according to Brachman (1986) as follows:
I- Exogenous and endogenous infections
II- Preventable and non preventable infection
III- Colonization, subclinical and clinical infections.

I- Exogenous and endogenous infections:

a) Exogenous infections:
These result from transmission from external source to patients through the following ways
1- Air borne.
2- Water and food.
3- Inoculation either through contaminated blood, contaminated infusion fluids.

4- Contact.

Hospital staff may act as a source transmitting potential pathogens between patients or from the environment to patients (Weber & Rutala, 1997).

b) Endogenous infections:

These occur when the causative organism is present as part of the patient’s flora (Brachman, 1986).

II-Preventable and non-preventable infections:

a) Preventable infections:

These are the infections which could have been avoided if an event related to the infection had been altered, e.g. hand washing before handling an intravenous device may prevent transmission of gram negative organisms from the hands of personnel to the intravenous insertion site. Nosocomial infection that are most preventable are caused primarily by medical devices used for patients care (Woeltje and Fraser, 1998).

About one third of infections classified as nosocomial are potentially preventable (Horan et al., 1999).

The preventive measures were achieved in hospitals that established very effective infection control programs including adequate numbers of qualified staff and particular types of surveillance, reporting and control methods (Whitehouse et al., 1998).
b) Non preventable infections:

These are the infections which occur regardless of all preventable strategies, e.g. immunocompromised patients who develop gram negative septicemia from their own flora (Swartz, 1994).

III- Colonization, subclinical and clinical infections:
a) Colonization:

It is the presence and multiplication of microorganisms in or on a host with no clinical manifestation or detectable immune response

b) Subclinical infections:

When the host and microorganisms interact to cause a detectable immune response.

c) Clinical infections:

When sufficient damage is done resulting in clinical signs and symptoms (Brachman, 1986).

Risk factors for nosocomial infection:

These factors which increase susceptibility to nosocomial infections may be general or specific.

I-General factors:

1-Age:

It has been demonstrated that the elderly have a disproportionate share of nosocomial infections (Wenzel, 1985).

Rebollo et al., (1996) found that nosocomial infections incidence is two fold greater in patients older than 65 years.
Outbreaks of health care-acquired infection can be devastating in neonatal intensive care units. Neonates are especially susceptible hosts because of prematurity of organ systems, low birth weight, and the use of invasive devices (Hass and Treza, 2002).

2-Chronic diseases:

Patients suffering from chronic diseases such as malignancy, diabetes mellitus, cardiovascular diseases, hypogammaglobulinemia and deficient cellular immunity are highly susceptible to infection (Schaberg et al., 1991).

3-Drugs:

a- Corticosteroids: patients receiving steroids are susceptible to infections because steroids can cause depression of antibody formation, diminished phagocytic capacity of leucocytes and/or suppression of capillary formation (Rebollo et al., 1996).

b- Antibiotics: antibiotics alters the normal bacterial flora, select the resistant strains of the organisms (Schaberg et al., 1991).

4-Obesity:

Obesity is a significant risk factor for clinical relevant nosocomial infections (Chobain et al., 1995).

II-Specific factors:

A-Risks for nosocomial urinary tract infection:

1-Sex:

Females are at a higher risk of infection (Stamm, 1991).
2-Instrumentation:

One in every 10 patients admitted to hospital receives a catheter and three quarter of hospital acquired urinary tract infection (HAUTI) are related to catheter (Wilson, 1995).

The duration of bladder catheterization is an important risk factor, the risk of infection rises from 19% for 5 days catheterization to 50% for 14 days catheterization (Villers, 1990). Cystoscopy may predispose to UTI (Stamm 1991).

B-Risks for nosocomial wound infection:

1-Duration of pre operative hospitalization:

The infection rate associated with 1.5 day pre-operative stay was 6%, while 2 days or more of pre-operative hospitalization lead to 14.7% infection rate (Krukowski & Matheson, 1988).

2-The length of operation:

The operation of long duration (7 hours) was observed to increase post-operative wound infection (Simchen et al., 1984).

3-Pre-operative hair removal:

The rate of infection was 3.1% when shaving with a razor immediately prior to surgery, 7.1% when shaving in the 24 hours prior to surgery and 12% when shaving more than 24 hours prior to surgery (Cruse and Foord, 1980).

4-Adequacy of blood supply:

Facial wounds are less prone to infections than the upper extremity wounds, but these are in turn less vulnerable to infection than lower extremity wounds (Hardy, 1981).
5-Urgency of the operation:

Urgent operations carried a higher risk of infection. This may be because they are usually performed on more critically ill patients, more frequently lead to bleeding and account for more re-operation (Krukowski, Matheson, 1988).

6-Drains:

These are risk factors, since they provide a portal of entry for microorganisms (Magee et al., 1976).

7-Blood transfusions:

Excessive blood transfusions have been considered as predisposing factor to surgical site infection (Rebollo et al., 1996).

8-Time of day of operation:

The rates of infection were found to be higher from 7:30 p.m to 12 midnight. However, this could be due to a disproportionately high number of non clean and non elective operations during the evening and night (Simechen et al., 1984).

C-Risks for nosocomial pneumonia:

1- Mechanical ventilation and respiratory equipment:

Patients who received respiratory therapy have a high incidence of pneumonia by more than 7% (Cross and Roups 1981). This increased risk is partially due to carriage of oropharyngeal organisms upon passage of endotracheal tube into the trachea during intubation, contaminated respiratory equipment, as well as depressed host defenses (Tablan et al., 1997).
2-Surgery:

Three quarters of hospital acquired pneumonia have been found in patients who have undergone surgery (Haley et al., 1985). This may be due to impairment of the defenses of the respiratory tract by endotracheal intubation and anaesthetic gases, also aspiration is more likely to occur during anaesthesia (Johansen et al., 1980).

3-Nasogastric tubes:

Nasogastric tubes increase the reflux of fluid from the stomach providing a route for bacteria to migrate from the stomach to colonize the oropharynx and cause pneumonia (Craven et al., 1991).
PATIENTS AT RISK IN AN INTENSIVE CARE UNIT

Introduction:

Infection is one of the principal hazards to which patients in critical care centers are exposed. In addition to being more susceptible, they are exposed to greater hazards of contamination and cross infection than most patients in ordinary wards. This is due to they receive much more nursing attention and handling and various forms of instrumentation in particular tracheostomy, mechanical ventilation, aspiration of bronchial secretion, catheterization of urinary tract, treatment of open wounds and prolonged intravenous infusion (Lowbury et al., 1992).

Nosocomial infection are endemic and epidemic in ICUS and represent a significant source of morbidity, mortality and cost. Infections that develop in the critical care environment account for 25% of all nosocomial infection and rates are five to ten times higher than in general words (Wenzel, 1983).

Nosocomial infection rates vary between hospitals and according to the type of critical care unit. Rates of nosocomial infection are high in burn unit patients, low in coronary care unit and two to nine times as high in surgical critical care unit patients (S.C.C.U) as in medical critical care unit (M.C.C.U) patients with the exception of pneumonia which occur more in M.C.C.U than in S.C.C.U. Susceptibility to infection is also related to the length of stay in critical care unit (Craven et al., 1988).

The interval between admission and development of infection in the first 5 days is similar in S.C.C.U and M.C.C.U but more S.C.C.U patients develops nosocomial infection between 5th day and 25th day,
is due to the fact that S.C.C.U patients are subjected to invasive devices more frequently than M.C.C.U and due to long duration of hospitalization after admission (Maki, 1986).

The commonest microorganisms causing disease in critical care units are gram negative bacilli and staphylococci. Gram negative bacilli encountered in critical care units include: Enterobacteriaceae (E. coli, Klebsiella spp., Proteus spp., Enterobacter spp., and Serratia spp.), Pseudomonas aeruginosa, Hemophilus influenza and Bacteroids fragilis. These organisms are known as opportunistic pathogens, that they are normally harmless commensals, but they are able to multiply in tissues and cause diseases in immunocompromized patients (Atherton & White, 1983). These organisms of low virulence become invasive and cause serious illness, because these patients are immunocompromized, several procedures are carried out in the critically ill patients which repeatedly breaches in the mucous membrane and increase the likelihood of local infection (Stoddart, 1988).

Sources of infection in ICU:

Sources of infections could be exogenous, endogenous and cross infections.

1-Exogenous infection:

Exogenous source may be from the introduction of the equipment used to treat the patient. Invasive devices are often needed for management of critically ill patients, but the risk of nosocomial infection is increased and directly related to the length of time the device is in place. Almost all of nosocomial infection are device related (Craven et al., 1988).
2-Endogenous infection

Critical care patient is at great risk from his own intestinal flora, the transmission of such colonization is possible in patients with primary gasterointestinal disease, such as postoperative wound infection, pelvic abscess, and peritonitis (Atherotn and White 1978). The stomach was shown to act as a source of the gram negative bacteria colonizing the lungs. In patients under artificial ventilation, reflux of gastric contents leading to colonization of the oesophagus, oropharynx and tracheostomy site (Atherton & white, 1983). Similarly the patient’s intestine may be the source of endogenous infections. For example Pseudomonas aeruginosa is present in intestine in about 20% of surgical patients (Allen et al., 1987). Although it is harmless in the intestinal lumen of healthy persons, it is responsible for pneumonia, urinary infection, intraabdominal sepsis and septicaemia in critically ill patients (Stoddard et., 1982).

3-Cross infection:

Cross infection is the transfer of a microorganism from one human to another, this source may be another patient, member of a staff or a visitor (Casewell & Philips, 1977).

Patient to patient transfer by contact route is usually indirect and through contaminated objects. The types of organisms transmitted on solid objects will depend on the total time of exposure of the organism to dry conditions and on the size of initial inoculum (Parker, 1984).

The hands of staff are an important vehicle specially for transient microorganisms and that hand washing makes significant contribution to the control of HAI (Larson, 1988). The most important microorganisms
spread by hand contact are S. pyogenes and gram negative bacilli (Burnie, 1986).

Types of Infections in ICU:

The ICU patients are at risk for eight types of nosocomial infection:

I- Pneumonia.
II- Intravascular catheter related infections.
III- Wound infections.
IV- Urinary tract infection (UTI).
V- Nosocomial bacteremia.
VI- Sinusitis.
VII- Antibiotic associated colitis.
VIII- Ventriculitis or meningitis from intracranial pressure (ICP) monitors (Wenzel et al., 1983).

I-Pneumonia:

Pneumonia is the most common fatal HAI occurring in 9% to 21% of patients requiring mechanical ventilation with an associated mortality rate of 20% to 80%. Nosocomial pneumonia represents a significant proportion of all HAI. It account for about 25% of all HAI and result in a mortality ranging from 25-35% (DalNogare, 1994). Nosocomial pneumonia in ICU represents a major threat to the recovery of patients with respiratory failure receiving mechanical ventilation (Gianfranco, 1990).

* Sources of infection:

Infection may be acquired from:

1- The hands of medical personnel.
2- Contaminated respiratory equipment.
3- Contaminated fluids and medications introduced into the respiratory system (Yannelli & Gurevich, 1988).
4- Spread from the patient’s endogenous flora, mostly in GIT. This is due to the rapid colonization of aerobic gram negative rods in the oropharyngeal region of severely ill hospitalized patients (Hayland & Mandell, 1992).

*Pathogenesis of infection:*

Aspiration of bacteria directly from the oropharynx or around the artificial airway are important in the pathogenesis of nosocomial pneumonia. This occur more in patients receiving mechanical ventilation through an endotracheal tube, about two fold more than without respiratory device. (Cross & Roupe, 1981).

Infection occurs when the patient aspirates minute quantities of respiratory secretions, because of decreased cough and sneeze reflex, impaired mucociliary transport, or intubation that allows secretion to enter the lung around the tube (Castle & Ajemian, 1987).

This infection begins with colonization of upper respiratory tract by pathogenic microorganisms that then aspirated into the lung (Palmer, 1987).

Pneumonia results when a particular virulent organism reaches the lower airways and overcome the local lung defenses. A local inflammatory response follow with accumulation of neutrophils and other inflammatory cells in the peripheral bronchi and alveolar spaces (Niederman and Fein, 1988).
*Causative organisms:*

Gram negative bacilli were the predominant organisms isolated from 61% of cultured patients who developed ventilator associated pneumonia. *Pseudomonas aeruginosa* (31%), *Klebsiella pneumoniae* (18%), *Acinetobacter anitratus* (16%) and *Serratia marcescens* (13%) (*Craven et al., 1991*).

Species of bacteria other than gram negative bacilli, such as *Streptococcus pneumoniae*, *S. aureus*, and *Haemophilus influenzae* were isolated from the remaining patients. Viruses are responsible for some of the cases of nosocomial pneumoniae; *Respiratory syncytial, Parainfluenza and Influenza viruses* predominate in large number of cases (*Craven and Steger, 1992*).

* Johanson et al., (1988) explained that the polymicrobial nature of many nosocomial pneumoniae might be an important factor in the poor response of these infections to seemingly effective therapy aimed at the "predominant organism". A large number of patients who don’t have bacterial pneumoniae are exposed to expensive and ineffective antibiotics that also increase the risk for colonization with potentially pathogenic organisms and superinfection (*Gianfranco, 1990*).

II-Intravascular catheter related infections:

The increasing practice for intravascular devices, made usually of various plastics, into the body of patients coupled with the selection pressure of prevailing antibiotic use, has shifted the spectrum of significant microorganisms commonly encountered in diagnostic laboratories (*Peter, 1988*).
The predominant microorganisms are coagulase negative staphylococci (CNS) particularly, S. epidermidis causing more than 14% of nosocomial blood stream infections (Hamory, 1987). Other colonizing organisms are S. aureus and other skin organisms such as Corynebacteria, Streptococci, various gram negative aerobic bacilli, yeasts and filamentous fungi. S. epidermidis strains associated with such infections appear to produce greater quantities of extracellular slime than strains from other sources. This has the property of binding the bacteria to the polymer surface and interfering in a variety of ways with the body’s response to infection (Peter, 1988).

a-Infection in patients with endocardial pacemaker:

Infections are the most common complications linked to the use of cardiac pacemakers. The incidence of infection after pacemaker implantation has been reported to vary from 1-12.6%, mostly caused by Stapylococci with S. epidermidis isolates predominating over S. aureus (Peter et al., 1981).

S. aureus is mostly involved in “early onset” infections whereas S. epidermidis causes predominately “late-onset” infections (Choo et al., 1981).

b-Heart-catheter associated infection

The major complications associated with long term right arterial (Hickman) catheters are infections, both local and systemic and thrombosis occasionally associated with septic thrombophlebitis (Schuman et al., 1985). Early infections seen in patients with Hickman catheters is often local, involving the exit site, the catheter tunnel, or the surgical incision itself, septic thrombophlebitis and thrombosis as well as
catheter related sepsis tend to occur late in the life of a catheter (*Al-sibai et al., 1987*).

S. epidermidis is the predominant microorganisms encountered in cardiac catheter related infections. It is responsible for about 54% of cases, other organisms causing catheter sepsis including S. aureus (20%), Candida species (7.1%), pseudomonas aeruginosa (5.9%) and diphtheroid (4.7%) (*Press, 1984*).

**c-Intravascular cannula related infections**

The introduction of plastic cannulae allowed infusions to be given via the same vessel for much longer periods than with steel needled, but it has also the difficulties of bacterial colonization and resultant infections (*Maki et al., 1996*).

Colonization of intravascular cannulae may remain silent or be associated with a simple fever, if it is caused by the more pathogenic organisms, especially S. aureus, however it may result in septicemia or disseminated infection (*Bone, 1991*). The main hazard of prolonged intravenous cannulation is catheter sepsis in which the microorganisms multiply in fibrin clot adhering to the tip of the catheter and signs of acute septicaemia appear (*Parker, 1984*).

*Caustive organisms:*

According to *Widmer (1997)* organisms commonly encountered are:

- S. epidermidis accounts for 60% of CNS
- S. aureus frequently isolated from intra-venous (I.V) drug abusers and patients with total parenteral nutrition
• Gram positive bacilli that cause clinical infection are almost cultured from neutropenic patients.
• Gram negative bacilli are frequently isolated from ICU patients
• Fungi, especially candida spp., prolonged broad spectrum antibiotics, ‘oral’ parenteral nutrition and ‘haemodialysis’ are risk factors for candida catheter associated septicaemia.

(i) Peripheral intravenous catheter:
Peripheral intravenous catheters may be the most commonly medical devices used in many hospitals. Intravenous cannulae were placed in 80.6% of all hospitalized patients (Tager et al., 1983).

Examination of day specific phlebitis rates revealed a highly significant trend toward increased phlebitis rate from one day of catheterization to 5 or 6 days of catheterization (CDC, 1981).

(ii) Central venous catheters:
The rate of bacteraemia with use of central venous catheter ranges from 3.8% to 21% (Lindbaled & Wolff, 1985). Signs of inflammation are rare at the site of insertion of central venous lines, catheter associated infection is considered synonymous with colonization of > 15 colony forming unit (CFU) of semi quantitative culture method of the catheter tip. As with other vascular catheters that penetrate the epidermis, duration of catheterization was significantly associated with the increased colonization of the catheter with bacteraemia (Prager & Silvar 1984).

(iii) Peripheral arterial pressure monitoring catheters:
Peripheral arterial catheters used for measuring intra-arterial pressure and for monitoring arterial oxygenation are among common
devices in an intensive care setting, the rate of bacterial colonization ranges from 0.85 to 20% of catheters (Pinilla et al., 1983).

The rate of cannula culture positivity increases with increasing duration of cannulation especially after the fourth day (Damen et al., 1985).

(iv) **Pulmonary artery catheters:**

Insertion of balloon-tipped cardiac catheters for monitoring cardiac output and pulmonary artery pressure is quite common in today’s critical care areas, the rate of colonization ranged from 2.1 to 33%. The majority of organisms isolated were staphylococcus species, predominately S. epidermidis, with a scattering gram negative organisms including Pseudomonas and Acinetobacter. Candida species was rare (Donowitz, 1986).

(v) **Intra-aortic balloon pumping:**

Placing a catheter into the abdominal or thoracic aorta as a blood pressure assistance device has gained wider usage, infections complications of such devices are related primarily to the presence of local wound infection. Bacteremia complicates 0.8% of these procedures, but the rate of local wound infection was 2.4% (Pennington et al., 1983).

**III- Wound Infections:**

Accurate diagnosis of wound infection depends on a thorough knowledge of the patient’s history, physical examination and bacteriological information from gram stain and wound culture. Nosocomial wound infection can develop after both clean and contaminated surgical procedure (Olson and Lee, 1990).
The incubation period for wound infection from S. aureus is usually 4 to 6 days. These infections are usually well localized and are characterized by thick, creamy, odorless pus (Lively and Pruitt, 1990).

Gram negative wound infections usually from contamination with enteric contents and therefore, may be accompanied by anaerobic streptococci or bacteroides fragilis if the large bowel was the target of operation or injury. The incubation period for most of these organisms is 7 to 14 days (Lively and Pruitt, 1990).

**IV-Urinary Tract Infections (UTI):**

*Incidence:*

Urinary tract infections are the commonest type of infection acquired in ICU. It accounts for 30-40% of all such infections (Willson, 1995).

*Sources of infections:*

The bedding of patients, urinals, bed pans sinks and hands of the nurses are known to harbor a large number of the microorganisms (Speller to Humphreys, 1998).

*Predisposing factors:*

The major predisposing factor is the presence of an indwelling urethral catheter or urologic instrumentation as cystoscopy and prior exposure to broad spectrum antimicrobial therapy (Garibaldi, 1993). Nosocomial UTI occurs in ICU patients, because these patients are usually seriously ill and are usually catheterized for longer periods of time, as part of intensive care package as in patients with spinal injury (Ohkawa, 1990). It is likely that mechanical and chemical irritation of
urethral and bladder mucosa by the catheter enhances the susceptibility of the structures to adherence by microorganisms (Wilksch et al., 1983).

Another predisposing factor is the colonization of the perineum with microorganisms from the intestinal tract especially gram negative bacteria. When a urethral catheter is in place, the bacteria which colonize the perineum travel along the outside of the catheter into the bladder. This route accounts for 70% of episodes of bacteruria in women (Daifuku & Stamm, 1984).

Gram negative bacilli predominate in the perineal flora of the long-term catheterized patient and are frequently recovered from their urine (Kunin & Steel, 1985).

* Causative microorganisms:

Harding et al., (1991) reported that out of 15 isolates from urinary catheter related infections: there are 9 isolates of E. coli (60%), 2 Enterobacteria (13.5%), Klebsiella (6.75%), Pseudomonas aeruginosa (6.75), and Enterococci (13.5%).

Also Ohkawa (1990) stated that Pseudomonas aeruginosa is responsible for 16% of urinary catheter related infections followed by Klebsiella 6%, E. coli 6%, Candida species 6% and Staphylococci 8% (Ohkawa, 1990).

* Mechanisms of infection:

Bacteria enter the bladder of catheterized patient in one of three ways:

1- They may be introduced with the catheter at the time of insertion.
2- They may travel along the outside of the catheter from gram negative bacilli in the perineal flora of long term catheterized patients.

3- They may travel along the inside lumen of the catheter, as a result of cross infection from enteric bacteria carried on to hands of staff when the catheter is disconnected or handled (Daifuku & Stomm, 1984).

*Diagnosis:*

In non catheterized individual, the diagnosis of UTI is usually based on clinical symptoms. In catheterized individual, frequency and dysuria will not be apparent and other symptoms may be absent, particularly in the elderly or confused patient. The diagnosis of UTI may be confirmed by microbiology laboratory through isolation of 10 organisms or more per/ml from a urine specimen (Koziol & Henderson, 1994).

**V-Nosocomial Bacteremia**

A nosocomial bacteremia is defined as a clinically important blood culture positive for a bacterium or fungus that is obtained after more than 48 hours following hospitalization (Garner et al., 1988).

Nosocomial bacteremia have been divided into two categories, primary and secondary (Garner et al., 1988). Primary bacteremia occur without any recognizable focus of infection with the same organism at another anatomic site (urine, lung, wound) at the time of positive blood culture (Schaberg et al., 1991).

Episodes of bacteremia secondary to intravenous or arterial lines are typically classified as primary bacteremia (Banerjee et al., 1991). Secondary bacteremia is the infection that develop subsequent to a
documented infection with the same microorganism at another body site
(Schaberg et al., 1991).

Causative organisms:

Organisms responsible for noscomial primary bacteremia are S. aureus, enterococci, candida spp., E. coli, Enterobacter species, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas aeruginosa and Streptococcus (Schaberg et al., 1991).

Major sources responsible for secondary bacteremia are respiratory tract, genitourinary tract, gastrointestinal tract and surgical wounds (Pittet and Wenzel 1995).

S. aureus secondary bactermias commonly complicate infections of the respiratory tract, intravenous devices related infections and infected haemodialysis fistulae. The rate of bacteremia complicating nosocomial UTI varied with the pathogen, being highest with serratia marcescens lowest with S. epidermidis (Kreiger et al., 1983).

* Risk factors:

General risk factors for bacteremia:

A-Host underlying conditions (Bone, 1992; Pittet et al., 1996).

1-Extremes of age
2-Underlying disease
3-Severity of illness
4-Malnutrition
5-Host susceptibility
B-Microbiologic factors (Pittet et al., 1994).
   1-Type of microorganism (Virulence)
   2-Bacterial inoculum
   3-Host colonization process
   4-Anti microbial resistance

C- Therapeutic factors (Pittel et al., 1996).
   1-Length of hospital ICU stay
   2-Indwelling devices
   3-Invasive procedure
   4-Adequacy for therapy for primary infection

D-Environmental factors (Taunton et al., 1994; Fridkin et al., 1996):
   1-Admission to ICU.
   2-Nurse to patient ratio
   3-Under staffing or overcrowding

* Specific risk factors to nosocomial pathogens:

1- Coagulase-negative Staphylococci (CNS) (Gray et al., 1995):
   Indwelling devices, central venous lines, length of stay, intravenous lipid emulsions, severity of acute illness and low birth weight.

2-Methicillin resistant staphlococcus aureus (MRSA) (Piettet et al., 1996):
   Severe underlying disease, confined to bed, poor clinical prognosis, prolonged hospital stay and prior antibiotic therapy.
3-Enterococci (Montecarlo et al., 1994):

Severe underlying disease, recent major surgery, ICU admission, prolonged hospital stay, prior exposure to antibiotics, exposure to more than five antibiotics, cephalosporine use, haematologic malignancy, gastrointestinal colonization, and receipt of vancomycin or antianaerobic drugs.

4-Candida species (Piettet et al., 1999)

Candida colonization, recent major abdominal surgery, ICU admission, prolonged hospital stay, prior exposure to antibiotics, haemodialysis, transfer from another hospital, diarrhea, low birth weight and prematurity.

VI- Sinusitis:

Sinusitis accounts for at least 5% of ICU nosocomial infections. Risk factors include intubation, nasogastric tubes and facial trauma (Caplan and Hoyt, 1982).

VII- Antibiotic-associated colitis:

Clostrtidium difficile can be cultured in up to 21% of hospitalized patients. The nature of nosocomial infection is increasing. Antibiotics suppress the endogeneous flora and the C. difficile proliferate (Tedesco, 1982).

Diagnosis is confirmed by fecal assay for C. difficile toxin. Vancomycin, metronidazole and bacitracin have been used as primary treatment. To curtail the emergency of vancomycine resistance enterococci (VRE) vancomycin should not be used as the 1ry treatment in ICUs (McFarland et al., 1989).
VIII-Ventriculitis or meningitis from intracranial pressure (ICP) monitors:

Ventricular catheters are commonly placed to diagnose and aid in the management of increased intracranial pressure. The nosocomial infection rate for ventriculitites is reported between 0% and 27%. Etiology of infection is believed to be either contamination at time of insertion or catheter contamination after insertion. The risk factors for ventricular related infections were:

1- Duration of ventricular catheter if more than 5 days.

2- Irrigation of the system.

3- Intracranial pressure over 20 mmHg.

4- Intracranial haemorrhage with intraventricular hemorrhage.

5- Neurosurgical operations. (Mayhall et al., 1984).
ANTI MICROBIAL DRUG RESISTANCE
IN THE INTENSIVE CARE UNIT

Introduction:

The development and use of antibiotics for bacterial infections is one of the most remarkable accomplishments in medicine of the 20th. However, antibiotics resistant bacteria were found in clinical isolates soon after the introduction of the earliest antimicrobial agents resistance have been emerged in common pathogens in every part of the world and problems of resistance are still presently serious among the immunocompromised host. The most important of these organisms are methicillin resistant staphylococcus aureus, penicillin resistant streptococcus pneumoniae, vancomycin resistant enterococcus, and certain gram negative bacilli due to extended spectrum beta lactamase production. These antibiotic resistance have made antimicrobial therapy of many infections extremely difficult or virtually impossible in some instances (Yamaguchi and Ohno, 2001).

The widespread use of broad spectrum antibiotics has led to emergence of antibiotic resistant organisms this suggests that a policy of administering limited duration, narrow spectrum antibiotics may reduce drug resistance. This problem is particularly serious in critically ill patients, especially those with ventilator associated pneumonia (Franklin et al., 2002).

Extensive antibiotic resistance has developed in gram negative bacteria due to both innate resistance in some species and the fact that they are highly adapt at acquiring antibiotic resistant determinants from each other.
Antibiotic resistance develops through the following three basic mechanisms, alteration of the drug target, prevention of drug access to the target (including actively removing the drug from the bacteria) and drug inactivation. Certain gram negative microorganisms are particular problems in the ICU, including Pseudomonas aeruginosa, Acinetobacter spp, Stenotrophomonas maltophilia and the Enterobacteriaceae are the combination of an increasing population at risk, and the natural virulence and adaptability of gram negative bacteria guarantees that critical care physicians will face a persistent and increasing challenge from these pathogens (Waterer and Wunderink, 2001).

Surveillance of antibiotic resistance is especially important in ICU because the infection rates are much higher there than in other hospital wards and most epidemics with multiresistant bacteria originate in ICU (Hanberger et al., 1999).

Choice of anti-microbial therapy:
1-Identification of the infecting microorganism:
   
   This can be performed by rapid methods such as Gram stain of normally sterile fluids including cerebrospinal, ascitic, pleural, or articular fluids.

   The gram stain of faeces allows one to visualize the presence of leukocytes and to differentiate diarrhea caused by invasive microorganisms such as Salmonella, Shigella, Campylobacter, and EIEC, from other (such as toxin-related) etiologies.

   Immunologic methods and molecular biology techniques such as PCR have expanded the diagnostic possibilities, allowing the identification of virus and uncommon bacteria.
However, final identification requires culture methods, therefore, it is important to obtain samples for culture before starting any therapy.

After initiations of therapy, cultures are often sterile even with viable microorganisms in the host (Moellering et al., 1995).

2-Determination of the susceptibility of the microorganism:

It is the second step in selecting the proper antibiotic agent. The most common is the disk diffusion method, described by Bauer et al. (1966). This is an easy and inexpensive method but provides semiquantitative information (Rosenblatt, 1987). A variant of the diffusion test, the E-test, has been introduced. The E-test uses diffusion of a continuous concentration gradient of an antibiotic from a plastic strip into an agar medium to yield quantitative measurement of antibiotic susceptibility.

Quantitative measurement of susceptibility had been classically performed by dilution testing; by varying drug concentration in a series of test tubes or wells.

The broth dilution test gives quantitative data on the drug concentration required to inhibit the organism. The minimum inhibitory concentration (MIC) represents the lowest concentration of the antibiotics that prevent visible growth after an 18 to 24 hours incubation period (Gomez-Herruz et al., 1995). It is possible to determine the concentration of drug that kills the test strain, the minimal bactericidal concentration (MBC), which represents the lowest concentration of antibiotics that totally suppresses growth or results in a 99.9% decline in colony count after overnight incubation (Eligonads & Moellering, 1991).
Table (1): Emerging antimicrobial resistance problems in nosocomial pathogens.

<table>
<thead>
<tr>
<th>Organism group</th>
<th>Type of antimicrobial resistance</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphy lococci (coagulase-negative &amp; S.aureus)</td>
<td>Meticillin</td>
<td>Altered penicillin binding proteins</td>
<td>(Jones et al., 1994; Tóen et al., 1993).</td>
</tr>
<tr>
<td></td>
<td>Quinolone</td>
<td>Altered DNA gyrase</td>
<td></td>
</tr>
<tr>
<td>Enterococci</td>
<td>B-lactam drugs</td>
<td>Altered penicillin-binding proteins, B-lactamase</td>
<td>(Tóen et al., 1995; Jones &amp; Cormican, 1996).</td>
</tr>
<tr>
<td></td>
<td>Glycopedptides</td>
<td>Aminoglycoside modifying enzymes</td>
<td></td>
</tr>
<tr>
<td>Streptococcus Pneumoniae</td>
<td>Penicillin</td>
<td>Alterations in penicillin-binding proteins</td>
<td>(Applebaum ;1992)</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemolyticus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leuconostoc Species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram-negative bacilli</td>
<td>Extended spectrum/ B-lactam drugs B-lactamase inhibitor combinations</td>
<td>Extended spectrum B-lactamases &amp;amp B-lactamases; reduced permeability.</td>
<td>(Quintallini &amp; Courvalin, 1995; Pfaller &amp; Herwaldt, 1997).</td>
</tr>
<tr>
<td></td>
<td>Quinolones</td>
<td>Transposon (Tn 21) mediated resistance Altered DNA gyrase</td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td>Polynes and azoles</td>
<td>Reduced permeability: altered target; over-expression of target efflux pump</td>
<td>(Iwen et al., 1995) (Pfaller &amp; Herwaldt 19987).</td>
</tr>
<tr>
<td>Aspergillus species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida krusei</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida histaniae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida glabrata a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichosporon beigelli</td>
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</tr>
</tbody>
</table>

3-Host factors:

The third issue important for selecting the proper antibiotic is the adaptation of the therapy to the host. Factors that should be taken into account are as follows: previous history of adverse side effects, age,
presence of genetic or metabolic abnormalities, pregnancy, renal and liver function, and the site of infection (Philpson, 1983).

**Causes of the emergence and dissemination of antimicrobial resistance:**

a- The excessive and inappropriate use of antimicrobials is the principle cause of the emergence of resistance. Antibiotic therapy produces marked effects on the host’s endogenous flora and exerts selective pressure in favor of resistant microorganisms (Dellinger et al., 1994). The resistance becomes more prevalent when clinicians rely on the latest, most potent broad-spectrum agents for prophylaxis and treatment of infections. Initiation of antibiotics before proper evaluation and obtaining samples for culture (Goldman et al., 1996).

Increasing numbers of critically ill and immunocompromised patients are now being treated in hospitals and because these patients are susceptible to a broad range of pathogens, physicians are more likely to administer broad-spectrum agents for empirical treatment of presumptive infection (Goldman and Huskins, 1997).

b- Inconsistent application of basic infection control techniques by hospital personnel (e.g. caregivers neglect hand washing and wearing gloves before and after patient contact) which leads to dissemination of resistant strains as it occurs primarily via person to person transmission (Ehrenkranz and Alfonso, 1991).
Examples Of Drug Resistant Pathogens

A) Methicillin–Resistant staphylococcus aureus (MRSA)

Epidemiology:

S. aureus is a major pathogen that is associated with serious community acquired and nosocomial disease (Emori et al., 1993). In the United Kingdom, S. aureus is the second most common isolate from blood cultures after E. coli and is by far the most common hospital acquired organism (Anonymous, 1997).

The rate of mortality from invasive S. aureus disease was high and the introduction of penicillin had a dramatic impact on treatment, the semisynthetic penicillin, methicillin was introduced in 1959 to overcome the problems that arose from the increasing prevalence of penicillinase producing isolates of S. aureus resistant to penicillin G and penicillin V. MRSA strains rapidly emerged and became a major clinical problem within hospitals during the 1960s in Europe and the 1970s in the United States and elsewhere (Grubb et al., 1998).

S. aureus is a common cause of soft tissue infection and cause osteomyelitis, arthritis, bacteremia with metastatic infection and scalded skin and toxic shock syndromes. CNS have became increasingly important cause of nosocomial bacteremia associated with invasive monitoring, intravascular catheters and prothetic heart valves or joints. Most staphylococci produce β-lactamase and are resistant to penicillin. An increasing proportion of S. aureus have intrinsic resistance to methicillin (MRSA), are present major problems in hospitals for the control of cross infection (Marchese et al., 2000).
The initial MRSA isolates were resistant to multiple drugs including penicillin, tetracycline, and usually streptomycin. Sometimes they were resistant to erythromycin, lincomycin, neomycin, kanamycin, and novobiocin (Jepson, 1986). The second wave MRSA isolates are usually resistant to several antibiotics which often include gentamicin, trimethoprim, and more recently, ciprofloxacin and mupirocin (Lin Zhi-Mei et al., 1994). S. aureus is part of the normal flora of the skin. MRSA may replace sensitive strains of S. aureus on the skin, which it will colonize without causing infection but provide a reservoir from which it may spread to other patients or staff (Murder et al., 1991).

Controlling of MRSA is possible if strict measures are taken before the organism become endemic (Kotilainen et al., 2003).

Clinical Significance:

S. aureus causes a variety of infections ranging from mild infection of the skin, boils and abscesses, to serious systemic infections; septicemia, pneumonia, and major wound infection. MRSA causes the same type of infection as sensitive strains of S. aureus and most studies suggest that it has equal pathogenicity (French et al., 1990).

The virulence of MRSA varies but usually is greater than that of methicillin susceptible strains. (Duckworth, 1993).

Mechanism of resistance:

The determinant of methicillin resistance is the mec A gene, which encodes the low affinity penicillin binding protein, PBP 2a. MRSA carries the methicillin resistant gene mec A on its chromosome (Dejonge
et al., 1992). This gene is distributed worldwide and is probably transposable (Kreiswirth et al., 1993).

Multiple episodes of horizontal transfer and recombination may have contributed to the spread of resistance determinants in natural populations (Musser and Kapur, 1992). Some spread may have occurred between the transposones of the organisms, and new clones have emerged, but cross infection of organisms in colonized or infected patients is the main mode of spread (Boyce et al., 1994). There is enormous variation in the phenotypic expression of methicillin resistance, with many strains showing heterogenous resistance (Hartman and Tomasz, 1986).

**Mode of transmission:**

The main mode of transmission of MRSA is via hands of healthcare workers which may be contaminated by contact with:

a- Colonized infected patients,

b- Colonized or infected body sites of personnel themselves, or

c- Devices or environmental surfaces contaminated with body fluids containing MRSA. However, other modes of spread (eg. Via the air) can occur (Ayliffe, 1996).
B) Vancomycin-Resistant Enterococci

**Epidemiology:**

The incidence of vancomycin resistance among Enterococci and Enterococcus faecium in particular has increased sharply in the last few years and these organisms have become labeled as the nosocomial pathogens of the 1990s (Pegues et al., 1997).

By 1994 and 1995, 41% of all NNIS hospitals reported at least one nosocomial enterococcal infection, and a recent report by Edmond et al., (1996) notes that attributable mortality is approximately 40%. All this concern directed toward the rapid emergence of VRE because of the different problems it comprises including:

1- The lack of available antimicrobials for therapy of VRE infections as VRE are resistant nearly to all other available agents and this results in a serious therapeutic dilemmas (CDC, 1993).

2- The potential emergence of vancomycin resistance in clinical isolates of S. aureus and S. epidermidis is a serious public health concern (Edmond et al., 1996).

Outbreaks have involved multiple strains, indicating horizontal transfer via either plasmids or transposons. However inter and intrahospital spread of VRE strains with identical pulsed field gel electrophoresis (PFGE) profiles have also been observed indicating clonal transmission (Woodford et al., 1995).

The VanA gene, which is frequently plasmid borne and confers high resistance to vancomycin can be transferred in vitro from
enterococci to a variety of gram positive organism including S. aureus (Noble et al., 1992).

**Mechanism of Resistance:**

Glycopeptide antibiotics inhibit cell-wall synthesis by complexing with the D-alanyl: D-alanine (d-Ala: d-Ala) residues of the pentapeptide side chain of peptidoglycan precursors. This prevents the transfer of the precursors into the peptidoglycan molecule by transglycosidases (Arthur and Courvalin, 1993).

Resistance to glycopeptides in enterococci is phenotypically and genetically heterogenous, three genotypes have been described-VanA, Van B, and Van C (Woodford et al., 1993). Three phenotypes of vancomycin resistance (VanA, VanB, and VanC) have been described based primarily on susceptibility testing to vancomycin and teicoplanin (Arthur and Courvalin, 1993).

Table (2): Relationship between genotype and phenotype for VRE (Arthur and Courvalin, 1993; Hastings and Jolley, 1995).

<table>
<thead>
<tr>
<th>Property</th>
<th>Van A</th>
<th>Van B</th>
<th>Van C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin Susceptibility MIC (μg/L)</td>
<td>High-level Resistance &gt; or = 64</td>
<td>High- or low-level resistance &gt; or = 4</td>
<td>Low level resistance-2-32</td>
</tr>
<tr>
<td>Teicoplanin Susceptibility MIC (μg/L)</td>
<td>Resistant &gt; or = 16</td>
<td>Sensitive 0.5-1</td>
<td>Sensitive 0.5-1</td>
</tr>
<tr>
<td>Expression of resistance</td>
<td>Inducible</td>
<td>Inducible</td>
<td>Constitutive</td>
</tr>
<tr>
<td>Resistance gene Location</td>
<td>Plasmid</td>
<td>Chromosome*</td>
<td>Chromosome</td>
</tr>
<tr>
<td>Transferable</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Species showing each genotype</td>
<td>E. faecium</td>
<td>E. faecium</td>
<td>E. gallinarum</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>E. faecalis</td>
<td>E. casselliflavus</td>
</tr>
<tr>
<td></td>
<td>E. avium</td>
<td>E. avium</td>
<td>E. flavescens</td>
</tr>
<tr>
<td></td>
<td>E. durans</td>
<td>E. durans</td>
<td></td>
</tr>
</tbody>
</table>

* Plasmid-mediated transferable van B resistance has been described (Woodford et al., 1995)
Risk factors for VRE acquisition:

There are certain patient populations that have been found to be at increased risk for VRE or colonization such as:

1. ICU patients as it is thought to be the consequence of certain features specific to the intensive care setting: a high concentration of severely compromised patients, continued use of indwelling devices and invasive procedures; and widespread empiric use of antimicrobial agents directed against Gram negative bacilli (Tornieporth et al., 1996). Therefore, ICUs may serve as reservoirs for nosocomial pathogens and may enable them to spread throughout the hospital (CDC, 1995).

2. Patients in the oncology or transplant wards, and those who have an intraabdominal or cardio-thoracic surgical procedure or those with an indwelling urinary or central venous catheter (Boyce et al., 1995). They are usually critically ill, with severe underlying disease or immunosuppressed patients (Boyce et al., 1995).

3. Prolonged hospital stay, the median time for acquisition of VRE in a medical ICU in a hospital was 5 days (range from 3-5 days (Bonten et al., 1996).

4. Previous administration of parenteral multiantimicrobial and/or vancomycin is also involved in acquisition of VRE (Morris et al., 1995).

5. The abuse of vancomycin had been reported constantly as a risk factor for infection and colonization (Hastings and Jolley, 1995). However, restriction of vancomycin use didn't reduce the rate of colonization with VRE in a hospital where VRE strains were endemic (Morris et al., 1995).
Clinical significance:

The main pathogens in the genus are *E. faecalis* and *E. faecium*. Although they are normal inhabitants of the bowel they were increasingly reported as a cause of HAI especially in seriously ill patients (*Emori and Gaynes, 1993*).

Urinary tract infections: are the commonest enterococcal infection, they are responsible for 10% of UTI and 16% of nosocomial UTI (*Murray, 1990*).

Intra-abdominal wound and pelvic infections: are the next commonest cause of enterococcal infections (*Murray, 1990*).

Bacteremia: is the third type of infections, nosocomial enterococcal blood stream infections occur at rates of 3-4 per 10,000 hospital discharges (*Banerjee et al., 1991*). They are responsible for 10% of all infections acquired in hospitals (*Emori and Gaynes, 1993*).

*Landry et al. (1989)* reported that the direct mortality due to enterococcal bacteremia was 31% and that enterococcal bacteremia added 39 days to the hospital stay. Severe sepsis or septic shock, the most advanced stages of the inflammatory response to infection, developed in >80% of patients with VRE bacteremia (*Edmond et al., 1996a*).

Enterococci can cause endocarditis in adults, and may cause meningitis and bacteremia in neonates (*Murray, 1990*).

Since enterococci are part of the normal flora of gastrointestinal tract and female genital tract, most of infections have been attributed to the patient's endogenous flora (*Murray, 1990*).
Transmission:

Reports have demonstrated that enterococci including VRE, can spread by direct patient to patient contact, indirectly via transient carriage on hands of personnel, or via contaminated environmental surfaces and patient care equipment (Boyce et al., 1994). Taking into consideration that the nature of enterococci allows it to persist almost everywhere, they can be found in soil, food, water and animals (Free and Sahm, 1995).
C) Drug Resistant Gram Negative Bacilli

Despite the significant increase in the frequency of nosocomial infections caused by gram positive organisms, gram negative bacilli as a group remain frequent and important causes of nosocomial infections. The widespread use of broad spectrum antibiotics has led to emergence of antibiotic-resistant strains of many gram negative organisms. This problem is particularly serious in critically ill patients especially those with ventilator associated pneumonia. Extensive antibiotic-resistance has developed in gram negative-bacteria, due both to innate resistance in some species and the fact that they are highly adaptable acquiring antibiotic-resistant determinants from each other (Emori and Gaynes, 1993).

Gram-negative pathogens in the hospital environment all present natural resistance and the capacity to develop rapidly acquired resistance. Dissemination of resistance is due to either to dissemination of a colonel strain or dissemination of the gene of resistance amongst bacteria belonging to unrelated species. In medical practice, the development of resistance posses various problems: a therapeutic problem for the physician who must prescribe an active antibiotic that does not select resistant mutants, a microbiological problem in relation to the difficulty of detecting particular resistance such as the production of extended spectrum beta-lactamase, a problem for the infection control team whose task is to limit the dissemination of multi-resistant bacteria. Certain Gram-negative microorganisms are particular problem in ICU, including Pseudomonas aeruginosa, Acinetobacter spp, Stenotrophomonas maltophilia, and the Enterobacteriaceae. The combination of an increasing population at risk, and the natural virulence and adaptability of
gram negative bacteria guarantees that critical care physicians will face a persistent and increasing challenge from these pathogens (Waterer and Wunderink, 2001).

Beta lactams are the most widely used antibiotics, and beta-lactamases are the greatest source of resistance to them. Resistance of Enterobacteriaceae to cefazidime or cefotaxime implies extended-spectrum beta-lactamase (ESBL) production in E. coli and Klebsiella spp., especially if susceptibility to cephamycins (e.g. cefoxitin and cefotetan) or carbapenam (e.g. meropenem or imipenem) is retained. Depression of amp C beta lactamases in Enterobacter spp, and Citrobacter freundii is another important mechanism and can be inferred from cross resistance to beta lactamase inhibitor combinations and to all cephalosporins except fourth generation agents (Livermore and Brown, 2001).

The extended-spectrum B-lactamases (ESBLs) are increasing in frequency among klebsiella species and other Enterobacteriaceae such as E. coli and Citrobacter species and have been identified most frequently in teaching hospitals and intensive care units (Hernandez et al., 2003).

The ESBLs are located on transmissible plasmids and may be present with other resistance factors, resulting in multiresistant pathogens (Quintilian and Courvalin, 1995).

Organisms that produce ESBLs can disseminate easily in units (such as ICUs and transplantation units) that use broad-spectrum cephalosporins extensively. In some cases, a single bacterial strain may be transmitted from patient to patient; In other situations a resistance
plasmid may be transmitted among various bacterial strains (Neuwirth et al., 1996).

The resistant gene responsible for beta-lactamase can be carried on mobile genetic elements or transposons. Members of the Tn21-related transposons are capable of transferring a wide variety of resistance factors from organism to organism and are likely responsible for the development of many multiresistant Gram-negative bacilli that have been implicated in nosocomial outbreaks (Tenover, 1991).

An inducible chromosomal type cephalosporinase that confers resistance to almost all B-lactam drugs, including third-generation cephalosporins and B-lactamase-inhibitor combinations, can be produced by strains of Enterobacter, Citrobacter and Serratia species, indole positive Proteus species and P.aeruginosa that carry the Amp C gene (Chow et al., 1991).
D) Drug-Resistant Tuberculosis

Multi-drug resistant tuberculosis (MDR-T.B.) by definition is: tuberculosis resistant to two or more of the main line anti-tuberculous drugs (usually isoniazid & rifampicin with or without other drugs) (Cohn et al., 1997).

Drug resistant disease should be considered when there is (Cookson and Jarvis, 1997),

1- A history of previous incomplete or non-compliant treatment.
2- Contact with a patient with known drug resistant disease.
3- Disease probably acquired in a country with high incidence of drug resistance.
4- Disease not responding to treatment.

Maloney et al., (1995) stated that eight outbreaks of MDR-TB in the united states have been investigated by the CDC between 1990 and 1992. These outbreaks have been characterized by delayed diagnoses, inadequate treatment regimens, high mortality and significant rates of nosocomial transmission (Dooley et al., 1992).

Resistance in Mycobacterial tuberculosis:

Streptomycin (SM) was the first drug shown to be effective in the treatment of tuberculosis (Dooley et al., 1992). However monotherapy led to the selection of resistant strains of Mycobacterium tuberculosis (selection occurs when one or two cells in population of bacteria are naturally resistant to the antibiotic; these cells are able to survive and multiply and eventually the sensitive cells are replaced by resistant ones (Musser, 1995).
El Resistant Candida

The emergence of fungal pathogens such as Candida species as important agents of nosocomial bloodstream infections has resulted in increased use of established anti-fungal such as amphotericin B and newer classes of anti-fungal agents such as the triazoles have been developed (Epsinel-Ingroff and Pfaller, 1995).

Standardized anti-fungal susceptibility testing methods have demonstrated that fungi such as Candida lusitaniae and Aspergillus species may be relatively resistant to amphotericin B (Pfaller et al., 1997).

Barchies et al. (1995) reported that two outbreaks caused respectively by a strain of C. albicans resistant to fluconazole and a strain of C. lusitaniae resistant to amphotericin B and 5-fluocytosine; This should warn microbiologists and infection control personnel that multiresistant strains of candida may develop and spread within the hospital environment (Pfaller, 1995).

**Fluconazole resistance:** In vitro susceptibility testing has indicated that C. krusei is natively resistant to fluconazole (Wingard, 1994). It is of interest that the in vitro susceptibility assays indicating resistant to fluconazole suggested that the organism was susceptible to miconazole (an imidazole). Thus, resistance to one azole may not indicate resistance to other azoles (Biganardi et al., 1991).

**Polyene resistance** has been associated with an alteration in the sterol make up of the cell membrane (Dick et al., 1980).
Role Of The Microbiologic Laboratory In Detection Of Multi Resistant Organisms

Role of the microbiologic laboratory in detection of MRSA:

1-Disk diffusion susceptibility tests:

It is carried as recommended by the NCCLS (NCCLS,1990). The bacterial inoculum, \(5 \times 10^7\) cfu/ml, is plated on to Muller-Hinton agar (MHA), and disks containing oxacillin 1 ug are applied. The plates are examined after incubation at 35°C for 24h (NCCLS, 1990).

2-Broth macrodilution:

It is performed in Muller-Hinton broth (MHB) according to NCCLS. Oxacillin is tested at final concentration from 0.25 to 32 mg/L and with NaCl 2% w/v added to the broth. Incubation is done at 35°C for 24h (NCCLS, 1990).

3-Oxacillin and methicillin screening agar:

MHA is supplemented with oxacillin 6mg/L or methicillin 10mg/L and NaCl 4% w/v.

The inoculum is prepared in 2 ways either:

- As recommended by NCCLS Guidelines (1990) giving \(10^7\) cfu/plate or
- As suggested by Kloos and Jorgensen (1985) giving \(10^4\) cfu/plate.

The plates incubated at 35°C for 24h.

4-Methicillin agar screen:

50ul of frozen stock culture is inoculated into 2ml of trypticase soy broth (TSB), and incubated at 37°C in a shaking device that ensues
vigorou s aeration and a high bacterial concentration (10^9-10^{10} cfu/ml)
100ul of these cultures were then plated on the trypti case soy agar (TSA)
plates containing methicillin (25 mg / l.). Methicillin resistance was
confirmed by surface growth after incubation for 24-48h at 37°C
(Delencastre et al., 1991).

In general, addition of NaCl, a cooler incubation temperature (30-
35°C), neutral pH and prolonged incubation (48h) favor detection of
MRSA (Kloos and Jorgensen, 1985).

The inoculum size is critical when screening agar tests are used and
that exclusively dilute inocula produce false-negative results (NCCLS,
1990).

5-Automated systems:

Several automated systems have been evaluated for early detection
of MRSA. Among these systems, Microscan-WA and the Vitek
Automicrobic system have been the most popular (Knapp et al., 1994).

Although these instruments are good for susceptibility testing of
some bacteria, a high percentage of false negatives was found for the
detection of methicillin resistance among S. aureus (Waites et al., 1996).

6-Molecular methods:

The variability inherent in discriminating among strains by
phenotypic methods has stimulated interest in DNA-based typing method.
1- Dot-blot hybridization: with a gene probe internal to the mec A gene,
isolated as the Xba-PstI fragment recovered from plasmid PMF13,
which carries the mec A determinant (Resende and Figueiredo, 1997).

ii- REA of chromosomal DNA identifies the epidemic strains, but due to the numerous overlapping bands that are produced, it is difficult to interpret small variations in the restriction profiles (Resende and Figueiredo, 1997).

iii- PCR: Application of repetitive extragenic PCR and enterobacterial repetitive intergenic consensus sequence analysis for the discrimination of MRSA strains have proven to be useful (Lessing et al., 1995).

Ribosome spacer PCR (RS-PCR): Amplification of fragments of the 16S-23S rRNA intergenic spacer region by RS-PCR can detect a significant level of length and sequence polymorphisms at the genus and species levels. Analysis of these sequences has been useful in differentiating closely related members of a number of genera (Gurtler and Barrie, 1995).

Bezason et al., (1995) stated that a preliminary report has suggested that PCR-amplified fragments of the intergenic spacer regions of S. aureus can produce patterns which are strain specific and this technique would be suitable for use in local laboratories for typing MRSA isolates related to an outbreak.

iv- Pulsed field gel electrophoreses (PFGE): It has been recommended as a highly discriminatory method for typing of MRSA isolates because it can distinguish among several concurrent epidemic strains.
However, it is a time consuming and expensive typing method not well suited for screening large number of isolates by a diagnostic laboratory (Resende and Figueiredo, 1997).

Role of the microbiologic laboratory in detection of VRE:

The microbiology laboratory is the first line of defense against the spread of VRE.

* Antimicrobial susceptibility testing:

1-Disk diffusion:

According to NCCLS guidelines a standard disk diffusion testing is performed on Muller-Hinton agar by using commercially prepared 30ug vancomycin (NCCLS, 1993).

Glycopeptide antibiotics diffuse poorly through agar, and disks typically give small zones of inhibition. Consequently, the difference between the sizes of inhibitory zones for susceptible and resistant bacteria is not as great as that for some other antibiotics (Caveaghi et al., 1992). Therefore, disk diffusion method is not a reliable means of detecting all levels of vancomycin resistance (Woodford et al., 1995). It is effective for high level resistant entero cocci, these organisms usually show no zone of inhibition around 30ug vancomycin disks. Woodford et al. (1991) and Snell, (1994) reported that laboratories using 5ug disks of vancomycin were more likely to detect low level resistance than those using 30ug disks.

Isolates with low level resistance may be incorrectly categorized by disk diffusion as intermediate. Any growth haze appearing inside the zone of inhibition after prolonging the incubation period to 48 hours,
allows categorizing some low level resistance strains as resistant by disk diffusion (Woodford et al., 1995).

2-Dilution Methods (MICs Test):

Traditionally, agar dilution MICs have been the standard method used to compare the ability of other methods for detection of glycopeptide resistant organisms (Woodford et al., 1995).

Broth microdilution: using cation-adjusted Muller-Hinton Broth in accordance with NCCLS guidelines, correlate well with agar dilution

This method allows detection of low-level resistant organisms (Sahm and Olsen, 1990)

Broth microdilution has replaced agar dilution as the reference method in some studies (Tenover et al., 1993).

A clinically significant point for MIC determination is that the trough level of an antibiotic (i.e. it is the antibiotic serum level just before the next dose and this is a reasonable approximation of the antimicrobial activity in the tissues) should exceed the MIC (Catchpole and Hastings, 1995).

A dose of 500 mg of vancomycin achieves a trough level of 1.5mg/L and this is adequate for susceptible organisms only (Cunha, 1995).

3-E- Test (Epsilon test):

The E-test is able to detect all levels of vancomycin resistance in enterococci and appears to be more sensitive than disk diffusion for detecting strains for which intermediate vancomycin MIC were-8-16ug/ml (Schulz and Sahm, 1993).
4-Breakpoint screening:

The use of agar plates containing a single breakpoint concentration of glycopeptides might be regarded as ideal phenotypic method for diagnostic laboratories to screen for glycopeptide resistance (Woodford et al., 1995).

It was found that Muller-Hinton agar plate containing 6 or 8 ug of vancomycin per ml is reliable alternative to disk diffusion and it is simple to perform (Willey et al., 1992).

Swenson et al., (1994) reported that some studies suggested that single agar concentration tests was easier if brain-heart infusion(BHI) agar is used.

Snell (1994) stated that laboratories using breakpoint of vancomycin of 4 mg/L were more likely to detect low-level resistance.

Remel Synergy Quad plate is a commercially available agar screening method for detecting VRE, that is based on NCCLS recommended guidelines. The Remel product consists of BHI agar quadrants supplemented with vancomycin, gentamycin and streptomycin in concentrations of 6, 500, 2000ug/ml respectively (Free and Sahm, 1995).

The test is accurate and convenient to perform. Essentially, no matter how accurate the screening, the test is still a screen for which a positive results should be followed by further laboratory testing.

Media containing 10 ug/ml of vancomycin concentration were studied as a rapid method for detecting VRE at 10 ug/ml Enterococci
expressing clinically significant VanA and VanB phenotypes should grow, whereas those expressing the clinically less significant VanC phenotype wouldn’t (Stephen et al., 1994).

Several papers have described the utility of bile esculin and azide-based media supplemented with vancomycin for isolation of VRE a commercial agar [(bile esculin azide agar) with 6 ug of vancomycin per ml (BEAA)] was used in the initial screening step to establish relatively rapid (i.e., in ≤ 24h from the time of isolation) detection and characterization of VRE (VanHorn et al., 1996).

Most enterococcal isolates are detected on BEAA within 48h of inoculation but holding the plates for a full 72h increases the sensitivity. When growth is noted, Gram staining of growth on BEAA (i.e., to distinguish between interference by Gram+ve bacilli and the presence of Gram +ve cocci that could be VRE). When the presence of gram positive cocci is confirmed, isolates are subcultured on sheep blood agar plates to which a vancomycin disk (30 ug vancomycin) is added. Pyrazinamidase (PYR) testing is performed after overnight incubation (i.e., a negative PYR test indicates the isolate is not an enterococcus) while a positive test, coupled with the organism’s ability to grow on a BEAA plate containing 6 ug of vancomycin/ml, reliably classifies the organism as enterococci isolate (Free & Sahm, 1996).

The vancomycin inhibition zone size is then used to categorize VRE as Van A, Van B, or Van C. A buffer inhibition zone from > 6 to ≤ 15mm is recommended and the Van status of isolates that fall within this inhibitory zone range require confirmation based on phenotypic criteria such as vancomycin MIC determination. The MICs for Van A and Van B
stains, which typically have inhibitory zones of $\leq 14\text{mm}$, are $>16\mu\text{g/ml}$. Whereas, the MICs for vanC strains, which usually have inhibitory zones of $\geq 16\text{mm}$, are between 2& 16\mu\text{g/ml} (VanHorn et al., 1996).

5-Automated systems:

* The Vitek system:

Zabransky et al., (1994) stated that the detection of VRE by Vitek gram-positive susceptibility system (GPS) is an unacceptable method as false susceptibility still reported for some isolates.

Actually, to some extent the accuracy of the GPS depends on the level of resistance as all isolates with $\geq 128\mu\text{g/ml}$ are detected accurately by Vitek GPS, while detection of isolates with MIC $\leq 64\mu\text{g/ml}$ is sporadic (Free and Sahm, 1996). It was found that Vitek broth doesn’t sufficiently support expression of vancomycin resistance by certain strains to allow them to be detected by Vitek automated system (Jett et al., 1996).

If medium components are contributing to the problem, then manipulation of the vitek broth or selection of another medium such as BHI broth might enhance the ability of this system to detect vancomycin resistance (Jett et al., 1996).

* Microscan walkaway system:

It is one of the susceptibility testing instruments. It utilizes standardized microdilution trays that are read after overnight either photometrically or fluorometrically (Zabransky et al., 1994).

As vitek system, Microscan products also appear to be unable to detect all levels of VRE. However, visual examination of wells may
increase the sensitivity of some tests performed with these products (*Iwen et al., 1996*).

Fully automated methods may be unreliable in detecting low level resistance (VanB and VanC phenotype) (*Tenover et al., 1995*).

**6-Molecular Approaches:**

Molecular techniques, including gene probes and PCR are used now to detect antibiotic resistance genes. These techniques allow laboratories to determine the genotype of an antibiotic-resistant organism in a time equivalent to, or shorter than that required to perform a traditional susceptibility testing of the organism (*Woodford et al., 1995*).

PCR has been used for species identification of clinically relevant Enterococci (*Dutka-Malen et al., 1995*).

Species identification of *E. fecium*, *E. faecalis* and *E. gallinarum* relied on specific amplification of fragment intragenic to *ddlE. fecium*, *ddl E. faecalis* and *vanC-1* respectively (*ddl* is a chromosomally encoded D-ala-Dala ligase) (*Dutka-Malen et al., 1995*).

*E. casseliflavus* and *E. flavescens* are identified by specific amplification of *vanC-2*, *van C-3* respectively, and because of their extensive similarity, they are amplified by the use of the same primer (*Free and Sahm, 1996*).

Multiplex PCR-RFLP is a convenient rapid method for detecting glycopeptide-resistant Enterococci; that is because of the feasibility of
inoculating a single colony from a blood agar plate directly into the PCR mixture (Patel et al., 1997).

In conclusion, the strains are classified as non susceptible to vancomycin if any of the following criteria is met:
- A vancomycin MIC >4ug/ml by agar dilution
- A yield of vanA, vanB or vanC1 amplification product by PCR.
- Identification according to the scheme of Facklam and Collins of an isolate is either E. gallinarum or E. casselifavus (Free and Sahm, 1996).

**Role of the microbiologic laboratory in detection of extended spectrum β-lactamase (ESBL) producing gram negative bacilli:**

Routine testing may indicate that these organisms are susceptible to newer cephalosporins, but ESBL-and Amp C-producing organisms are not truly susceptible to these agents (e.g. cefotaxime, ceftriaxone, ceftazidime, and cefoperazone) (Pfaller and Herwaldt, 1997).

Moreover, many in-vitro susceptibility assays used in the clinical microbiology laboratory do not detect resistance unless the organism has been induced or the enzyme production is depressed (Emori and Gaynes, 1993).

In particular, rapid (3-to-5 hours incubation) automated or semiautomated methods and microdilution assays in which a relatively low inoculum of the organism (10^5 cfu/ml) is tested, are prone to make these very serious errors (McGowan and Metchock, 1995).
The laboratory must use a macrodilution broth method (1-to 5-ml total volume), an agar disk diffusion method, the E test, or a microdilution assay with sufficient inoculum (5x10^5 cfu/ml) and an incubation time of 18-24 hours to correctly identify organisms that carry these resistance determinants (Pfaller and Herwaldt, 1997).

National Committee for Clinical Laboratory Standards (NCCLS) has developed broth microdilution and disk diffusion screening tests using selected antimicrobial agents. Each Klebsiella pneumoniae, K. oxytoca, or Escherichia coli isolate should be considered a potential ESBL-producer if the test results are as follows:

Table (3): Diagnosis of ESBL by disk diffusion and MIC dilution method.

<table>
<thead>
<tr>
<th>Disc diffusion</th>
<th>MICs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefpodoxime ≤ 22mm</td>
<td>Cefpodoxime ≥ 2ug/ml.</td>
</tr>
<tr>
<td>Ceftazidime ≤ 22mm</td>
<td>Ceftazidime ≥ 2ug/ml</td>
</tr>
<tr>
<td>Aztreonam ≤ 27mm</td>
<td>Aztreonam ≥ 2ug/ml</td>
</tr>
<tr>
<td>Cefotaxime ≤ 27mm</td>
<td>Cefotaxime ≥ 2ug/ml</td>
</tr>
<tr>
<td>Ceftriaxone ≤ 25mm</td>
<td>Ceftriaxone ≥ 2ug/ml</td>
</tr>
</tbody>
</table>

The sensitivity of screening for ESBLs in enteric organisms can vary depending on which antimicrobial agents are tested. The use of more than one of the five antimicrobial agents suggested for screening will improve the sensitivity of detection. Cefpodoxime and ceftazidime show the highest sensitivity for ESBL detection.

If such strains are identified, the laboratory must undertake surveillance to determine the extent to which these organisms have spread within the hospital (McGowan and Metchock, 1995).
restriction fragment-length polymorphism with the IS6110 element (Wiid et al., 1994; Warren et al., 1996).

*Line Probe Assay:*

For rapid detection of rifampicin resistance (RMPR) of M. tuberculosis in clinical samples. After amplification of the region of the RNA polymerase, involved in RMPR, the amplified product is hybridized with a set of 10 oligonucleotides immobilized on to a membrane strip (DeBeenhouwer et al., 1995).

*Mutational analysis:*

Primers are synthesized to amplify small regions of the katG, inhA, rpoB, rrs and rpsL genes. A 50ng sample of DNA from each isolate is amplified in a 100ul reaction mixture and 5ul of the amplified products are subjected to SSCP analysis (Pretorius et al., 1995).

A mobility shift with regard to the M. tuberculosis H37Rv reference strain is scored as a mutation which is confirmed by restriction endonuclease digestion in cases where a restriction enzyme site is affected or by direct sequencing of the PCR product. Direct sequencing of PCR products is performed with the sequence PCR product sequencing kit according to the manufacturer's instruction (Debeenhouwer et al., 1995).

From the pattern obtained, the presence or absence of RMPR M.tuberculosis can be assessed in less than 48h (Debeenhouwer et al., 1995).
*PCR:

PCR is used directly on clinical samples to amplify genetic loci associated with rifampicin resistance (rpoB), and strain-specific polymorphisms (the direct repeat (DR) region) (*Goyal et al., 1997*).

Drug resistance was determined using a commercially available kit for detection of point mutation in the rpoB gene, and confirmed by nucleotide sequencing. Strain variation was determined using the spoligotyping method based on the presence or absence of variable linker sequences within the DR region (*Goyal et al., 1997*).

PCR-based tests for strain typing and for identification of rifampicin resistance provide important tools for identifying patients with MDR-T.B and for rapid monitoring of potential nosocomial spread of MDR-T.B. (*Goyal et al., 1997*).

Combination chemotherapy was shown to prevent the development of drug resistance (*Musser, 1995*). A combination of isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA) (the first-line drugs) is currently used with success in short course chemotherapy of tuberculosis (*Victor et al., 1997*).

Resistance to INH arises at a frequency of $10^{-5}$-$10^{-7}$ comparable to that reported for RMP (*Yew and Chau, 1995*) RMP is essential in short course chemotherapy regimens as, without it, treatment must be prolonged to 9 or 12 months to ensure sterilization of the lesions (*Yew and Chau, 1995*).
Role of microbiologic laboratory in diagnosis of fungal infection:

Fungal Susceptibility Testing:

NCCLS established the subcommittee for Anti-fungal Susceptibility Testing in 1982. Their studies led to the development of NCCLS document M2, titled “Reference Method for Broth Dilution Anti-fungal Susceptibility Testing of Yeasts”. This document was first published as M27-(P) reported in 1992 (NCCLS, 1992). It has been updated as M27-(T) entative (NCCLS, 1995).

Tornatore et al. (1996) reported that test conditions such as incubation time, buffer concentration, pH inoculum size, and endpoint determination criteria significantly affect results of susceptibility tests.

Table (4): Summary of the M27 methodology developed by NCCLS (NCCLS, 1995).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Implementation in the M27 methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methodology</td>
<td>1ml final volume or broth microdilution. 0.2ml final volume</td>
</tr>
<tr>
<td>Medium</td>
<td>RPM1-1640 containing 0.165 M Mops (PH, 7.0).</td>
</tr>
<tr>
<td>Fungal inoculum</td>
<td>0.5-2.5x10^7 organisms</td>
</tr>
<tr>
<td>Incubation time</td>
<td>48h (candida species) or 72h (cryptococcus neoformans)</td>
</tr>
<tr>
<td>Incubation temperature</td>
<td>35°C</td>
</tr>
<tr>
<td>Endpoint</td>
<td>Amphotericin B: optically clear tube: azoles and 5-fluorocytosine: 80% reduction in turbidity by comparison with growth control</td>
</tr>
<tr>
<td>Drugs and quality control (QC) isolates</td>
<td>Two QC isolates and corresponding QC ranges established via the M23 procedure are specified for amphotericin B, fluconazole, ketoconazole, itraconazole, and fluconazole.</td>
</tr>
</tbody>
</table>

MOPS= Morpholinepropanesulfonic acid.
STERILIZATION AND DISINFECTION

Sterilization and disinfection of patient care items are an important aspect of infection control. Every health care establishment should have a disinfection policy depending upon the services provided, types of items used and facilities available (Ayliffe, 1991).

Staff should be aware of the differences between sterilization and disinfection and the basic principles for selection of a procedure. The detailed procedure for treatment of various item should be available at word level.

1 - Sterilization:

Sterilization means freeing of an article from all microbes including bacteria, fungi and viruses as well as spores. It is required for all instruments and material coming in contact with tissues, body cavities or vascular system (Rutala, 1987).

Sterilization should be carried out in the Central Sterile Supply Department where quality control and monitoring can be effectively carried out (Block, 2001).

Sterilization is accomplished principally by steam under pressure (autoclaving), dry heat, by ethylene oxide gas or low temperature steam and formaldehyde (Shechmeister, 1991).

1 - Steam sterilization:

Steam sterilization is the most common and preferred method employed for sterilization of all items that penetrate the skin and mucosa,
Review of literature

providing that they are not damaged by heat and moisture. Steam sterilization is dependable, non-toxic, inexpensive, sporidical with rapid heating and good penetration of fabrics (Rutala et al., 1982).

*Method:
The steam must be applied for specified time so that the items reach a specified temperature.

121°C for 20 min at 1.036 Bar (15.03 psi) above atmospheric pressure.

134°C for 4 min at 2.026 Bar (29.41 psi) above atmospheric pressure (Rhodes et al., 1982).

2- Dry heat:
Dry heat is preferred for reusable glass, syringes ointments, powders and oils.

*Method:
A hot air oven equipped with fan or conveyor, which will ensure even distribution of heat. The recommended temperature and time for sterilization of medical equipment is as follows 170°C for 2h or 180°C for 1h.

Bacillus subtilis spores should be used to monitor the sterilization process for dry heat. The primary lethal process is considered to be oxidation of cell constituent (Rutala, 1995).

3- Ethylene oxide gas (ETO):
ETO is a colorless gas that is flammable and explosive. ETO has been widely used as a low-temperature sterilant since the 1950s.
It has been the most prevalent process for sterilizing temperature and moisture sensitive medical devices and supplies in health care institutions. The effectiveness of ETO sterilization is influenced by four essential elements: Gas concentration, temperature, humidity and exposure time. The operational ranges for each of these four parameters is 450 to 1200 mg/L, 29°C to 65°C, 45% to 85% and 2 to 5 hours respectively. ETO inactivates all microorganisms although the bacterial spores (especially B. subtilis) are more resistant than other microorganisms. For this reason, B. subtilis is the recommended biologic indicator. (Schneider, 1990).

The microbiocidal activity of ETO is considered to be the result of alkylation of protein, DNA and RNA. Alkylation, or the replacement of a hydrogen atom with an alkyl group, within cells prevents normal cellular metabolism and replication (Gross, 1995).

The main disadvantages associated with ETO are the lengthy cycle time, the cost, and its potential hazards to patients and staff; the advantage is that it can sterilize heat or moisture, sensitive medical equipment without deleterious results. The basic ETO sterilization cycle consists of five stages (preconditioning and humidification, gas introduction, exposure, evacuation and air washes) and takes approximately 2½ hours, excluding aeration time.

Table (5) characteristic of an ideal low-temperature sterilant (Schneider, 1994).

4-Vaporized Hydrogen Peroxide (VHP):

Hydrogen peroxide solutions have been used as chemical sterilants for many years. However, vaporized hydrogen peroxide was not
developed for the sterilization of medical equipment until the mid-1980s. One method for delivering VHP to the reaction site uses a deep vacuum to pull liquid hydrogen peroxide (30% concentration) from a disposable cartridge through a heated vaporizer and then, after vaporization, into the sterilization chamber (Schneider, 1994). New technologies such as VHP are attractive supplements. VHP offers several appealing features, including rapid cycle time (30 to 45 min), low temperature, environmentally friendly byproducts (H₂O, O₂), good materiel compatibility, and simple operation, instillation, and monitoring. VHP's limitations are that cellulose can't be processed, nylon became brittle, and the penetration capabilities are less than ETO (Gross, 1993).

5-Gas plasma:

Gas plasma have been referred to as the fourth state of matter (i.e., liquids, solids, gases, and gas plasma). And is a state of partially or fully ionized gas produced with the help of electromagnetic radiofrequency fields. Gas plasma are generated in an enclosed chamber under deep vacuum using radiofrequency or microwave energy to exit the gas molecules and produce charged particles, many of which are in the form of free radicals. A free radical is an atom with an unpaired electron and is a highly reactive species (Schneider, 1994).

Recently, low temperature sterilization methods using reactive plasma has been introduced which are not harmful for the environment and don’t produce toxic residues (Block, 2001).

The microbicidal effect of plasma is attributed to thermal dialing and chemical removal, to the biochemically reactive radicals which are capable of interacting with essential cell components (enzymes, nucleic acids) and disrupting the metabolism of microorganisms. Although this
method is safe for the environment and doesn't leave toxic residues, it is not suitable for porous items like paper and liner and also liquids. It has low diffusability and thus medical devices with long and narrow lumens can't be processed. There is also the potential to cause surface changes as erosion (Holler et al., 1993).

In the late 1980s, the first hydrogen peroxide gas plasma system for sterilization of medical devices (Sterrad) was field-tested. In this process the sterilization chamber is evacuated and hydrogen peroxide solution is injected and vaporized in the sterilization chamber after H₂O₂ vapor diffuses through the chamber (50 minute) and surrounds the items to be sterilized, radiofrequency energy is applied to the chamber to create a gas plasma. The process operates in the range of 37°C to 44°C and has a cycle time of 75 minute. The biological indicator used with this system is Bacillus subtilis spores (Alfa et al., 1996).

Another gas plasma unit (Plazlyte) differs from the sterrad unit in two ways. First, medical items in the plazlyte sterilizer one exposed only to a secondary gas plasma that has been characterized as less damaging to materials than direct exposure to the highly reactive (primary) gas plasma. This is accomplished by creating the gas plasma from the hydrogen, oxygen and argon gasses outside the sterilization chamber and then sending it into the chamber. Second, the plazlyte system uses a nonplasma, peracetic acid vapor (1mg/L) and hydrogen peroxide vapor (4mg/L) phase that is alternated with a plasma treatment. The biological indicator used with this system is Bacillus circulans (Holler et al., 1993).
Table (5): Summary of advantages and disadvantages for low temperature sterilization techniques *(Rutala, 1996)*

<table>
<thead>
<tr>
<th>Sterilization Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| Hydrogen peroxide Plasma sterilization (Sterrad) | *Safe for the environment and healthcare worker*  
*Leave no toxic residuals*  
*Cycle time is 75min and no aeration necessary*  
*Ideal for heat and moisture sensitive items since process temperature < 50°C Simple to operate, install (208 V outlet) and monitor* | *Cellulose (paper). Linens and liquids cannot be processed*  
*Sterilization chamber is small, about 3.5ft³*  
*Endoscopes or medical devices with lumen lengths >12 (31cm) or a diameter of <1/4 (6mm) cannot be processed at this time in the U.S.*  
*Requires synthetic packaging (polypropylene wraps, polyolefin pouches) and special container tray* |
| Plasma sterilization (AbTox) | *Safe for the environment and healthcare workers*  
*Cycle time depends on load and varies from 4h to 6h and no aeration necessary*  
*Ideal for heat and moisture sensitive items*  
*No corrosive effects and no harmful residues* | *Sterilization chamber is small, 55ft³*  
*No liquids or products harmed by vacuum can be processed*  
*Effectiveness not verified in peer-reviewed literature*  
*Limited to stainless steel surgical instruments (excludes lumen devices and hinged instruments) at this time* |
| 100% Ethylene oxide (ETO) | *Penetrates packaging materials, device lumens*  
*Single-dose cartridge and negative pressure chamber minimizes the potential for gas leak and ETO exposure*  
*Simple to operate and monitor*  
*Compatible with most medical materials* | *Requires aeration time to remove ETO residue*  
*Sterilization chamber is small, 4ft³ to 8.8 ft³*  
*ETO emission regulated by some states but catalytic cell removes 99.9% of ETO and converts it to CO₂ and H₂O*  
*ETO cartridges should be stored in flammable liquid states*  
*ETO is toxic, probable carcinogen and flammable* |
| 12% ETO/88% CFS | *Effective at killing microorganisms*  
*Penetrates medical packaging and many plastics*  
*Compatible with most medical materials*  
*Cycle easy to control and monitor* | *Some states (CA, NY, MI) require ETO emission reduction of 90-99.9%*  
*CFC (inert gas that eliminates explosion hazard) banned after 1995*  
*Potential hazards to staff and patients Lengthy cycle/aeration time*  
ETO is toxic, probable carcinogen and flammable |
| Peroxid acid | *Rapid cycle time (35-40min)*  
*Low temperature (50-55°C) liquid immersion*  
*Fully automated*  
*Environmentally friendly byproducts (acetic acid, O₂, H₂O)*  
*No adverse health effects to operators*  
*Compatible with wide variety of materials and instruments* | *Potential material incompatibility (i.e., aluminum anodized coating becomes dull)*  
*Used for immersible instruments only.*  
*Biological indicators may not be suitable for routine monitoring*  
*One scope or a small number of instruments can be processed in a cycle* |
II-Disinfection:

Disinfection describes a process by which pathogenic organisms with the exception of bacterial spores are killed, can be achieved by heat or chemicals. Chemicals used on inanimate objects are termed as disinfectants, whereas less toxic chemicals which can be used on the skin and living tissues are called antiseptics (Russel et al., 1982).

The efficacy of disinfection is affected by a number of factors, each of which may nullify or limit the efficacy of the process.

Some of these factors are prior cleaning of the object, the type and level of microbial contamination, the concentration and exposure time to disinfectant, the physical configuration of the object (e.g., cervices, hinges, lumens), and the temperature and pH of disinfection process (Favero and Bond, 1991).

Depending upon the spectrum of activity disinfectants can be graded as:

a- High level: i.e. effective against all vegetative bacteria fungi, viruses and mycobacterium but not high numbers of bacterial spores.

b- Intermediate level; which kill vegetative bacteria, mycobacteria, fungi and lipid viruses but with limited activity against non lipid small viruses and resistant spores.

c- Low level disinfectants are effective against vegetative bacteria but cannot be relied upon to kill mycobacteria, some of the viruses, fungi and have no action against resistant spores (Simmons, 1983).

Some high level disinfectants when used under strictly controlled condition eg: prolonged exposure time may act as sterilants (Ayliffe et al., 1993).
The simplest and the most effective method of disinfection is the use of moist heat, this is easily affected by boiling or the use of hot water and detergent. Chemicals should only be used when boiling is not possible and materials are heat sensitive (Russel et al., 1982).

**Selection of disinfection procedure:**

The selection of the procedure will depend on the degree of risk of infection involved in the use of the item. On this basis items are classified as critical, semi-critical and non critical (Rutala, 1987).

1. **Critical:** for instruments and other items that enter sterile tissues or vascular system including dental instruments, needles, scalpels, endoscopes. Sterilization is necessary, which can be achieved by steam under pressure if possible. If heat labile, the object may be treated with ethylene oxide or, if other methods are unsuitable, a chemical sterilant as 2% glutaraldehyde -based formulations, 6% stabilized hydrogen peroxide, and demand-relase chlorine dioxide (Favero, 1983).

2. **Semicritical:** for instruments that touch mucous membranes or broken skin. e.g. flexible endoscopes for the digestive and respiratory tract, laryngoscopes, endotracheal tubes, anesthesia equipment and vaginal specula. High level disinfection is required by use of wet pasteurization or glutaraldehyde, stabilized hydrogen peroxide, chlorine and chlorine compounds (Block, 1983).

Some semi-critical items (e.g., hydrotherapy tanks used for patients whose skin is not intact, thermometers) may require only intermediate level disinfection by use of chlorines, phenolics and iodophor (Russel et al., 1982).
3-Non critical: instruments that touch intact skin e.g. stethoscopes, tabletops, floors, bedpans, furniture, blood pressure cuffs. Usually cleaning with detergent and drying is adequate (Rutala, 1987).

Principles for use of disinfectants:

Heat is the best disinfectant. A chemical disinfectant should be used only when the item is heat labile.
- Disinfectant should be used as per manufacture’s instructions.
- Disinfectant should be diluted to the appropriate concentration by correct measurements and not approximation.

The container used for preparing the disinfectant should be sterile or high level disinfected.

The disinfectant should remain in contact with the surface of the article for a specific period of time (differ for different disinfectants). To allow for penetration into microbial cell, pre-cleaning of instruments before disinfection/sterilization is essential to reduce the bioburden and remove organic matter, which can serve to protect the organism from the action of disinfectant.

Staff carrying out pre-cleaning and disinfection must be trained in safe work practice and must wear protective gear like gloves, mask, eye protection and gown as appropriate (Block, 2001).

Factors affecting the activity of disinfectants and antiseptics:
1-Types of organisms:

Organisms vary greatly in their ability to withstand chemical and physical treatment. This variety is due to the biochemical composition of the cells and the protective mechanisms afforded, by the constituents. For example, spores have coats rich in proteins, lipids and carbohydrates as
well as cores rich in dipicolinic acid and calcium, all of which offer protection to spores. Cell wall of mycobacteria are rich in lipids which may account for their resistance to chemical and environmental stresses, particularly desication. By contrast, viruses containing lipid-rich envelopes are more susceptible to the effect of detergent and wetting agents (Kobayashi et al., 1984).

Schematic diagram showing the different types of organisms and the resistance to disinfectants

<table>
<thead>
<tr>
<th>Bacterial spores</th>
<th>Mycobacteria</th>
<th>Non Lipid viruses</th>
<th>Fungi</th>
<th>Bacteria</th>
<th>Lipid viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2-Number of organisms:

The disinfectant will be more effective when there are few microorganisms against which it has to act. Higher numbers of organisms require longer exposure time (Lynn, 1980).

3-Concentration of disinfecting agent:

The concentration of disinfecting agent is also important. Agents vary substantially among manufactures, and it is important that manufacture's instructions on preparation, dilution and use be followed carefully.

Proper concentration of infecting agents ensure the inactivation of target organisms and promote safe and cost-effective practices (Rutala, 1995).

4-Organic soil present:

Organic soil, such as blood, mucus, and pus affects killing activity by actually inactivating the disinfecting agent. In addition, by coating the
surface to be treated, organic soil prevents full contact between object and agent. For optimal killing activity, instruments and surfaces should be cleansed of excess organic material prior to disinfection (Cowen, 1978).

5-Nature of surface to be disinfected:

Certain medical instruments are manufactured of biomaterials that exclude the use of certain disinfection or sterilization methods because of possible damage to the instruments. An example is endoscopic instruments, which are readily damaged by the heat generated in an autoclave. Alternative methods must be used for this class of instruments (Rutala, 1995).

6-Temperature:

The activity of disinfectant is usually increased when the temperature at which it acts is increased (Bean, 1976).

The glutaraldehyde shows a very marked temperature dependent activity. The alkalinized form of this dialdehyde is more powerful agent at 20°C. However, at temperature of about 40°C, there is little deference in activity (Gorman et al., 1980).

7-Environmental pH:

pH can influence the activity of antimicrobial agent. Glutaraldehyde is more stable at acid pH, but more potent at alkaline pH, so alkaline activator must be added just before use (Russell et al., 1983).

8-Volume and dilution of disinfectant in use:

Large volumes are best and safer because it decrease the chance of inactivation and dilute contaminating organisms. Dilution of disinfectant must be with hot sterile D.W. and stored in sterile non-plastic container (Gorman et al., 1980).
Classes of disinfectants:

1- Alcohols (ethanol and isopropanol):

Alcohols have broad antimicrobial activity, ethanol and isopropanol at 70% are not sporicidal, their effectiveness being due to the inhibition of germinating spores (Diaz, et al., 2000).

Alcohols are the base for many other disinfectants for example, lysol spray contains 79% ethyl alcohol and only 0.1% orthophenylphenol, when used as surface spray or solution on inanimate objects, alcohol is an excellent pathogen destroyer; but it must be left in contact with the item to be disinfected for long periods to do its job-20 minutes contact time is considered proper for disinfection with ethyl alcohol. The higher the “proof” of an alcohol product, the better disinfectant it is, but the more volatile and evaporative it will be (Morton, 1983).

Isopropyl alcohol is not considered to be disinfectant-it’s main use is as a skin wipe to remove loose organic debris from the site of wound or infection (Diaz, et al., 2000).

* Mode of action:

Little is known about the specific mode of action of alcohols, but based on the increased efficacy in the presence of water, it is generally believed that they cause membrane damage and rapid denaturation of proteins, with subsequent interference with metabolism and cell lysis (Larson and Morton, 1991).

* Advantages:

Low cost, effective against many pathogens with correct contact time. Trauman et al., (2001) observed that strains of gram-negative bacteria were not regularly isolated from the hands of hospital personnel,
which had been disinfected regularly with alcohol before and after contact with patients.

* Disadvantages:

Long contact time required for disinfecting action, only certain types of alcohol contain true disinfectant properties; may dissolve synthetic surfaces, fumes may be irritating and contain a fire hazard risk, not effective against some viruses, evaporates quickly, so items being disinfected must be physically soaked alcohol to obtain disinfection.

2- Aldehyde:

(i) Glutaraldehyde

**BRAND NAMES:** Wavecide, Cidex, Sporocide, Banacide, Sterol:

This is a relatively new class of disinfectants which has come out within the past 25 years.

* Mode of action

The chemical action is to deactivate DNA and RNA proteins of microorganisms (*Clipsham, 1992*).

Glutaraldehyde used as high level disinfectant and chemical sterilant. Aqueous solution of glutaraldehyde are acidic and generally in this state are not sporicidal. Only when the solution is activated (made alkaline) by use of alkalinating agents to pH 7.5 to 8.5 does the solution become sporicidal. Once activated the solutions have a shelf life of 14 days because of the polymerization of the glutaraldehyde molecules at alkaline pH (*Russell, 1996*).

* Advantages:

Equally effective in water of any temperature and hardness, effective against essentially any pathogen even in presence of organic
debris. Solutions are good for longer periods than in any other disinfectant available with lowers cost per use. Speed of killing pathogens is very fast compared to other many disinfectants. It is available in many forms including sprays, concentrates and bulk, used for disinfection for materials that destroyed by heat and can used as sterilant if contact time (6-8h) (Schrimm et al., 1994).

* Disadvantages:

Eye and nasal irritant, may cause asthma and allergies, hence used in well ventilated area and container covered with well fitting lids. Eye protection and gloves should be worn while handling. It causes skin and mucous membrane irritation so, instruments must be rinsed before use (Bond, 1998).

(ii) Formaldehyde:

a-Formaldehyde solution (formalin):

It is used as a high level disinfectant and sterilant in liquid or in combination with low temperature steam. Formaldehyde is bactericidal virucidal and sporicidal, it act very slowly than glutaraldehyde (Power, 1995).

* Mode of action:

It is an extremely reactive chemical that interact with protein, DNA and RNA in vitro. It has long been considered to be sporicidal by virtue of its ability to penetrate into the interior of bacterial spores (Sykes, 1970).

Formaldehyde acts as a mutagenic agent and an alkylating agent by reaction with carboxyl sulphydryl, and hydroxyl group (Favero and Bond, 1991).
* Precautions for uses:
- It is potent eye and nasal irritant and may cause respiratory distress, eye irritant and skin allergy.
- Protective clothing such as gloves, eye protection and gowns should be worn.
- It must be used in well ventilated area. (Stewart et al., 1991; Rubber and Rutala, 1998).

b-Formaldehyde-releasing agents:

Several formaldehyde releasing agents have been used in the treatment of peritonitis. They include noxythiolin, tauroline, heramine. All of these agents are claimed to be microbicidal on account of the release of formaldehyde. However the antibacterial activity of taurolin, is greater than that of free formaldehyde, the activity of taurolin is not entirely the result of formaldehyde action (Kaufer and Masquardt, 1991).

OPA is a new type of disinfectant that is claimed to have potent bactericidal and sporicidal activity and has been suggested as a replacement for glutaraldehyde in endoscope disinfection (Alfa and Sitter, 1994).

OPA is an aromatic compound with two aldehyde groups. The mechanism of its antimicrobial action has been little studied, but preliminary evidence suggests an action similar to that of glutaraldehyde (Walsh et al., 1997).

3- Anilides:

The anilides have been investigated primarily for use as antiseptics, but they are rarely used in the clinic. Triclocarbon (TCC) is the most extensively studied in this series and is used mostly in consumer soaps.
and deodorant. TCC is active against gram-positive bacteria but significantly less active against gram-negative bacteria and fungi and lacks appreciable substantivity (persistency) for the skin. The anilides are thought to act by adsorbing to and destroying the semi-permeable character of the cytoplasmic membrane, leading to cell death (Hancock, 1984).

4- Biguanides

(i) Chlorhexidine

Chlorhexidine is the most used biocide in antisepic in particular in hand washing and oral products but also as a disinfectant and preservative. This is due to in particular to its broad-spectrum efficacy (Gardner and Gray, 1991).

Chlorhexidine is a bactericidal agent acts by damage to the outer cell layers followed by leakage intracellular constituents, high concentration of chlorhexidine causes coagulation of intracellular constituents (El-Moug et al., 1985).

Mycobacteria are highly resistant to chlorhexidine, and also chlorhexidine not sporicidal (Shaker et al., 1988).

(ii) Alexidine:

Alexidine differs chemically from chlorhexidine in possessing ethylhexyl end groups and has faster bactericidal effect (Chawner and Gilbert, 1989).

(iii) Polymeric biguanides:

Vantocil is a heterodisperse mixture of polyhexamethylene biguanides (PHMB). Polymeric biguanides have found use as general disinfecting agents in the food industry and very successfully, for the
disinfection of swimming pools. Vantocil is active against gram-positive and gram-negative bacteria although Pseudomonas aeruginosa and Proteus vulgaris are less sensitive. Vantocil is not sporicidal; PHMB is a membrane active agent that also impairs the integrity of outer membrane of gram-negative bacteria (Gilbert et al., 1990).

5- Halogen Releasing Agents:

Chlorine and iodine based compounds are the most significant microbicidal halogens used in the clinic and have been used for both antiseptic and disinfectant purpose.

(i) Chlorine-releasing agents (CRAs):

CRAs are highly active oxidizing agents and thereby destroy the cellular activity of proteins (Bloomfield, 1996).

Potentiation of oxidation may occur at low pH, where the activity of CRAs is maximal, although increased penetration of outer cell layers may be achieved with CRAs in the unionized state. Hypochlorous acid has long been considered the active moiety responsible for bacterial inactivation by CRAs (Dychdala, 1991).

CRAs at higher concentrations are sporicidal, this depends on pH and concentration of available chlorine (Russel, 1982).

The best known member of this class is sodium hypochlorite (bleach). Bleaches are very harsh but effective. They attack pathogens, organic debris and living tissues equally well. Bleach can create toxic fumes which can lead to chemical pneumonia, skin and eye irritation or burns (Bloomfield, 1996).

*Advantages:

Bleach is inexpensive, easily available without license depending on the concentration at which it is mixed it can kill most bacteria, viruses
and mycoplasmas, it is a potent deodorizer and works best in the presence of sunlight which releases more free radicals (which destroy cells including pathogens) (*Hoffman et al., 1981*).

**Disadvantages:**

It is very toxic to tissues and equipments, very rapidly inactivated by organic debris, it loses its effectiveness quickly while still on the shelf in the bottle. Bleach produces carcinogenic by-products and must be used in a well ventilated area, all objects treated with bleach must be well rinsed and allowed to dry (*Dychdala, 1991*).

(ii) Iodine and Iodophors:

Iodine is less reactive than chlorine, but is rapidly bactericidal, fungicidal, tuberculocidal, vruicidal and sporicidal (*Gottardi, 1991*).

Iodophor is a combination of iodine and a solubilizing agent or carrier, the resulting couple provides a sustained release reservoir of iodine and releases small amounts of free iodine in aqueous solution. The best known and most widely used iodophor is providine-iodine, this product and other iodophors retain the germicidal efficacy of iodine but, unlike iodine are generally nonstaining and are relatively free of toxicity and irritability (*Gottardi, 1991*).

Iodine penetrate into microorganisms and attacks key groups of proteins nucleotides and free fatty acids (*Rutala, 1995*).

6- Silver compounds:

The most important silver compound currently in use is silver sulfadiazine (AgSD). Silver and its compounds have long been used as antimicrobial agents. Silver metal, silver acetate, silver nitrate and silver protein, all of which have antimicrobial properties. In recent years silver
<table>
<thead>
<tr>
<th>CS</th>
<th>Chemicals</th>
<th>Initial Immune</th>
<th>2% Glutaraldehyde</th>
<th>Iodine</th>
<th>Phosphate 0.4-5%</th>
<th>Formamide 3-8%</th>
<th>Peroxide</th>
<th>Hydrogen Peroxide</th>
<th>Alcohol</th>
<th>Isopropyl Alcohol</th>
<th>Literature</th>
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<td>Literature</td>
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</table>

**Table (6)** Some common disinfectants with their use-dilution properties.
Problems with disinfection of health care equipment:

In some situations it is still difficult to choose a method of disinfection after considering the categories of risk to patients. This is especially true for a few medical devices (i.e., arthroscopes, laparoscopes, biopsy forceps) in the critical category because there is a controversy on whether we should sterilize or high level disinfect these patient-care items (Rutala, 1991).

Sterilization would not be a problem if these items could be steam sterilized, but most of these items are heat labile and sterilization is achieved by using ethylene oxide, which may be too time consuming for routine use between patients. Whereas new technology is making it easier to sterilize these items, evidence is lacking that sterilization of these items improves patient care by reducing the infection risk (Loffer, 1980).

This is also true for equipment in the semicritical category such as endoscopes, which may be heat labile and for which it may be difficult to expose organisms to sterilization process. For example, is the endoscope is used for upper gastrointesitinal tract investigation still a semi-critical item when it is used with sterile biopsy forceps or when it is used in a patient who is bleeding heavily from oesophageal varices? Provided that high-level disinfection is achieved, and all microorganisms except high numbers of bacterial spores have been removed from the endoscope, then the endoscope should not represent as infection risk and should remain in the semi-critical category (Miles, 1991).

Several other problems are associated with disinfection of patient-care items (Rutala, 1995).
Table (7): Inactivation of Hepatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV) by Disinfectants

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Concentration Inactivating 10^6 HBV in ST, 10 min, 20°C</th>
<th>Concentration Inactivating 10^6 HIV in ST, &lt;10 min, 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl alcohol</td>
<td>ND</td>
<td>50%</td>
</tr>
<tr>
<td>Glutaraldehyde</td>
<td>2%</td>
<td>ND</td>
</tr>
<tr>
<td>Glutaraldehyde-phenate</td>
<td>0.13% glut-0.44% phenol</td>
<td>ND</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>ND</td>
<td>0.3%</td>
</tr>
<tr>
<td>Iodophor</td>
<td>80 ppm</td>
<td>ND</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>70%</td>
<td>35%</td>
</tr>
<tr>
<td>Paraformaldehyde</td>
<td>ND</td>
<td>0.5%</td>
</tr>
<tr>
<td>Phenolic</td>
<td>ND</td>
<td>0.5%</td>
</tr>
<tr>
<td>Sodium hypochlorite</td>
<td>500 ppm</td>
<td>50 ppm</td>
</tr>
</tbody>
</table>

Abbreviations: ND, no data; ST suspension test (Martin et al., 1985).

Microorganism resistance to antiseptics and disinfectants

**Bacterial resistance to antiseptics and disinfectants**

In recent years, considerable progress has been made in understanding more fully the responses of different types of bacteria (mycobacteria, nonsporulating bacteria and bacterial spores) to antibacterial agents. As a result resistance can be either a natural property of an organism (intrinsic) or acquired by mutation or acquisition of plasmids (self-replicating, extrachromosomal DNA) or transposons (chromosomal or plasmid integrating, transmissible DNA cassettes). Intrinsic resistance is demonstrated by gram-negative bacteria, bacterial spores, mycobacteria and under certain conditions, staphylococci. Acquired, plasmid-mediated resistance is most widely associated with mercury compounds and other metallic salts. In recent years, acquired
resistance to certain other types of biocides has been observed, notably in staphylococci (McDonnell and Russell, 1999).

1. Intrinsic bacterial resistance mechanisms:

For an antiseptic or disinfectant molecule to reach its target site the outer layers of a cell must be crossed. The nature and composition of these layers depend on the organism type and may act as a permeability barrier, in which there may be a reduced uptake. Alternatively but less commonly constitutively synthesized enzymes may bring about degradation of a compound. Intrinsic (innate) resistance is thus a natural chromosomally controlled property of a bacterial cell that enables it to circumvent the action of an antiseptic or disinfectant. Gram-negative bacteria tend to be more resistant than gram-positive organisms, such as Staphylococci (Hugo, 1991).

(a) Intrinsic resistance of bacterial spores:

Bacterial spores of the genera Bacillus and Clostridium have been widely studied and are invariably the most resistant of all types of bacteria to antiseptics and disinfectants. Although Bacillus species are generally not pathogenic, their spores are widely used as indicators of efficient sterilization. Clostridium species are significant pathogens; for example, C. difficile is the most common cause of hospital-acquired diarrhea. Many biocides are bactericidal or bacteriostatic at low concentrations for nonsporulating bacteria, including the vegetative cells of Bacillus and Clostridium species, but high concentrations may be necessary to achieve a sporicidal effect (e.g., for glutaraldehyde and CRAs). By contrast, even high concentrations of alcohol, phenolics, QACs and chlorhexidine lack a sporicidal effect, although this may be achieved when these compounds are used at elevated temperatures (Bloomfield et al., 1990).
bilayer. In wild-type gram-negative bacteria, intact LPS molecules prevent ready access of hydrophobic molecules to phospholipid and hence to the cell interior. In deep rough strains which lack the O-specific side chain and most of the core polysaccharide, the phospholipid patches at the cell surface have their head groups oriented toward the exterior (George, 1996).

Gram-negative bacteria that show a high level of resistance to many antiseptics and disinfectants include P. aeruginosa, Burkholderia cepacia, Proteus spp. and Providencia stuartii. The outer membrane of P. aeruginosa is responsible for its high resistance; in comparison with other organisms, there are differences in LPS composition and in the cation content of the outer membrane (Bloomfield and Arthur, 1994).

Members of the genus Proteus are invariably insensitive to chlorhexidine, some strains that are highly resistant to chlorhexidine, OACs and diaminodines have been isolated from clinical sources. The presence of a less acidic type of outer membrane LPS could be a contributing factor to this intrinsic resistance (Cox and Wilkinson, 1991).

Few authors have considered peptidoglycan in gram-negative bacteria as being a potential barrier to the entry of inhibitory substances. The peptidoglycan content of these organisms is much lower than in staphylococci, which are inherently more sensitive to many antiseptics and disinfectants. Nevertheless, there have been instances where gram-negative organisms grown in sub-inhibitory concentrations of a penicillin have deficient permeability barriers (Crow, 1992).
2- Acquired Bacterial Resistance Mechanisms:

As with antibiotics and other chemotherapeutic drugs, acquired resistance to antiseptics and disinfectants can arise by either mutation or the acquisition of genetic material in the form of plasmids or transposons, it is important to note that “resistance” as a term can often be used loosely and in many cases must be interpreted with some prudence. This is particularly true with MIC analysis. Unlike antibiotics “resistance” or an increase in the MIC of biocide does not necessarily correlate with therapeutic failure (Alfa and Sitter, 1994).

(a) Plasmid-mediated antiseptic and disinfectant resistance in gram-negative bacteria.

Occasional reports have examined the possible role of plasmids in the resistance of gram-negative bacteria to antiseptics and disinfectants. Observed that plasmid RP1 did not significantly alter the resistance of Pseud. aeruginosa to QACs, chlorhexidine, iodine or chlorinated phenols, although increased resistance to hexachlorophene was observed. This compound has a much greater effect on gram-positive than gram-negative bacteria, so that it is difficult to assess the significance of this finding. Transformation of this plasmid (which encodes resistance to carbenicillin, tetracycline, neomycin and kanamycin) into E. coli or Pseud. aeruginosa did not increase the sensitivity of these organisms to a range of antiseptics (Alquarashi et al., 1996).

(b) Plasmid-mediated antiseptic and disinfectant resistance in staphylococci.

MRSA strains are a major cause of sepsis in hospitals throughout the world, although not all strains have increased virulence. Many can be referred to as “epidemic” MRSA because of the case with which they can
spread. Patients at particularly high risk are those who are debilitated or immunocompromised or who have open sores (Cookson and Philips, 1988).

Staphylococci are the only bacteria in which the genetic aspects of plasmid-mediated antiseptic and disinfectant resistant mechanisms have been described. In S. aureus these mechanisms are encoded by at least three separate multidrug resistance determinants. Increased antiseptic MICs have been reported to be widespread among MRSA strains and to be specified by two gene families (qacAB and qacCD) of determinants. The qacAB family of genes encodes proton-depentant export proteins that develop significant homology to other energy-dependent transporters such as the tetracycline transporters found in various strains of tetracycline-resistant bacteria. The qacA gene is present predominantly on the pISK family of multiresistance plasmids but is also likely to be present on the chromosome of clinical S.aureus strains as an integrated family plasmid or part there of. The qacB gene is detected on large heavy-metal resistance plasmids. The qacC and qacD genes encode identical phenotypes and show restriction site homology: the qacC gene may have evolved from qacD (Khunkiti et al., 1997).

(c) Plasmid-mediated antiseptic and disinfectant resistance in other gram-positive bacteria:

Enterococcus faecium strains showing high level resistance to vancomycin, gentamicin, or both are not more resistant to chlorhexidine or other non antibiotic agents. Furthermore, despite the extensive dental use of chlorhexidine, strains of Streptococcus mutans remain sensitive to it. To date therefore there is little or no evidence of plasmid-associated
resistance of non staphylococcal gram-positive bacteria to antiseptics and disinfectants (Anderson et al., 1997).

Hospital isolates of gram-negative bacteria are invariably less sensitive to disinfectants than other laboratory strains. Sub-inhibitory antibiotic concentrations may cause subtle changes in the bacterial outer structures, thereby stimulating cell-to-cell contact; it remains to be tested if residual concentrations of antiseptics or disinfectants in clinical situations could produce the same effect (Hiraishi et al., 1995).

Viral resistance to antiseptics and disinfectants

Potential viral targets are the viral envelope, which contains lipids and is a typical unit membrane; the capsid, which is principally protein in nature; and the genome. An important hypothesis was put forward in 1963 and modified in 1983 in which it was proposed that viral susceptibility to disinfectants could be based on whether viruses were "lipophilic" in nature, because they possessed a lipid envelope (e.g., herpes simplex virus or "hydrophilic" because they did not (e.g. poliovirus). Lipid-enveloped viruses were sensitive to lipophilic-type disinfectants, such as 2-phenyphenol, cationic surfactants (QACs). Chlorhexidine, and isopropanol, as well as ether and chloroform. Viruses are classified into three groups A (lipid containing), B (nonlipid picornaviruses), and C (other nonlipid viruses larger than those in group B) and disinfectants into two groups, broad spectrum ones that inactivated all viruses and lipophilic ones that failed to inactivate picornoviruses and parvoviruses (Klein and Deforest, 1983).

Conflicting results on the actions of disinfectants on different virus types were often reported and they suggested that the structural integrity of a virus was altered by an agent that reacted with viral capsids to
increase viral permeability, thus a "two-stage" disinfection system could be an efficient means of viral inactivation while overcoming the possibility of multiplicity reactivation, to explain an initial reduction and then an increase in the titer of disinfectant-treated bacteriophage. Multiplicity reactivation as a mechanism of resistance that clumping of poliovirus following partial inactivation by hypochlorite significantly increased the phage titer. It is envisaged as consisting of random damage to the capsid protein or nucleic acid of clumped, noninfectious virions from which complementary reconstruction of an infectious particle occurs by hybridization with the gene pool of the inactivated virions (Khunkitti et al., 1997).

Another resistance mechanism also involves viral aggregation e.g., the persistence of infectivity of formaldehyde-treated poliovirus and the resistance of Norwalk virus to chlorination. A typical biphasic survival curve of enterovirus and rotavirus exposed to peracetic acid is also indicative to the presence of viral aggregates (Harakeh, 1987).

Finally, there remains the possibility of viral adaptation to new environmental conditions. In this context, the development of poliovirus having increased resistance to chlorine inactivation. Clearly, much remains to be learned about the mechanism of viral inactivation by and viral resistance to disinfectants (Bates et al., 1977).

**Fungal resistance to antiseptics and disinfectants**

In comparison with bacteria, very little is known about the ways in which fungi can circumvent the action of antiseptics and disinfectants there are two general mechanisms of resistance (i) intrinsic resistance, a natural property or development of an organism and (ii) acquired resistance. In one form of intrinsic resistance, the cell wall presents a
barrier to reduce or exclude the entry of an antimicrobial agent. The evidence to date is somewhat patchy, but the available information links cell wall glucan, wall thickness, and relatively porosity to the susceptibility of *Saccharomyces Cerevisiae* to Chlorhexidine.

**Protozoal resistance to antiseptics and disinfectants**

Intestinal protozoa, such as *Cryptosporidium parvum, Entamoeba histolytica*, and *Giardia intestinalis*, are all potentially pathogenic to humans and have a resistant, transmissible cyst (or oocyst for cryptosporidium). Of the disinfectants available currently, ozone is the most effective protozoan cysticide, followed by chlorine dioxide, iodine, and free chlorine all of which are more effective than the chloramines. Cyst forms are invariably the most resistant to chemical disinfectants. The reasons for this are unknown, but it would be reasonable to assume that cysts, similar to spores, take up fewer disinfectant molecules from solution than do vegetative forms (*Jarroll, 1988*).

Some studies have compared the responses of cysts and trophozoites of *Acanthamoeba castellanii* to disinfectant used in contact lens solutions and monitored the development of resistance during encystation and the loss of resistance during excystation. The lethal effects of chlorhexidine and of a polymeric biguanide were time and concentration dependent, and mature cysts were more resistant than precystation trophozoites or precystation cysts. The cyst wall appeared to act as a barrier to the uptake of these agents thereby presenting a classical type of intrinsic resistance mechanism. *Acanthamoebae* are capable of forming biofilms on surfaces such as contact lenses. Although protozoal biofilms have yet to be studied extensively in terms of their response to disinfectant, it is apparent that
they could play a significant role in modulating the effects of chemical agents (Gray et al., 1995).

**Prion resistance to disinfectants**

The transmissible degenerative encephalopathies (TDEs) form a group of fatal neurological diseases of humans and other animals. TDEs are caused by prions, abnormal proteinaceous agents that appear to contain no agent-specific nucleic acid, an abnormal protease-resistant form of a normal host protein is implicated in the pathological process (Gray et al., 1999).

Prions are considered highly resistant to physical and chemical agents although the fact that crude preparations are often studied means that extraneous materials could, at least to some extent mask the true efficacy of these agents. There is currently no known decontamination procedure that will guarantee the complete absence of infectivity in TDE-infected tissues processed by histopathological procedures. Prions survive acid treatment but a synergistic effect with autoclaving plus sodium hydroxide treatment, is observed. Formaldehyde, unbuffered glutaraldehyde (acidic pH), and ethylene oxide have little effect on infectivity although chlorine-releasing agents (especially hypochlorites), sodium hydroxide, some phenols, and guanidine thiocyanate are more effective. With the information presently available it is difficult to explain the extremely high resistance of prions, save to comment that the protease-resistant protein is abnormally stable to degradative process (Ernst and Race 1993).
INFECTION CONTROL PROGRAM IN AN ICU

Introduction:

Prevention and control of nosocomial infection is of enormous importance not only to the affected patients but also to the finances of the health service as a whole (Department of Health, 1995).

Patients are protected against infection in hospital by a system of methods, the purpose of which can be summarized under three headings:
1- To remove the sources and reservoirs of infection this includes treatment of infected patients as well as sterilizing, disinfecting, and cleaning of contaminated materials and surfaces (Ayliffe et al., 1990).
2- To block the routes of transfer of bacteria from these sources and reservoirs to uninfected patients which include isolation of infected and susceptible patients, barrier nursing, (Bowell, 1992).
3- To enhance the patient’s resistance to infection-e.g. during operations, by careful handling of tissues and removal of sloughs and foreign bodies; also by enhancing the general defenses, as by control of diabetes, reinforcement of immunity to tetanus, and the use of antibiotic prophylaxis if and when this is indicated (Garner, 1996).

Prevention and control of hospital acquired infection is the function of infection control program.

Objectives of the infection control program (Seto, 1995).
1- Establish a surveillance program for nosocomial infections; monitor wards and clinics for incidence of infection to have an early warning system of increased or dangerous infection.
2- Investigation and management of an outbreak.

3- Maintain a good standard of cleanliness, disinfection and sterilization within the hospital.

4- Isolation of infected patients; isolate infections which are virulent or communicable, or which have antibiotic resistance of serious clinical concern.

5- Monitor standards of nursing and medical care to maintain sound sterile techniques and adherence to isolation precautions.

6- Monitor the health of employees to limit opportunities of infection spread between staff and patients.

7- Deal with any other matters in the hospital which may influence control of infection such as disposal of waste and laundry.

8- Provide an education program of staff so that they know their responsibilities in matters of infection control.

Establishment of Infection Control Program:

I-Surveillance of nosocomial infections:

A surveillance program, which includes the collection of data on infections, analysis to determine the significance and to identify any factors which may prevent infection, is necessary for an effective infection control program (Emmerson et al., 1996).

The purpose of infection control surveillance is to establish a database that describes when nosocomial infections or other noninfectious adverse events occur and how they are distributed. Therefore, it can be used to assess the quality of care in the hospital and improve patient care. Further, it sometimes leads us down paths that indirectly aid in the understanding of the causes of nosocomial infections and can be used to reduce nosocomial infection rates (Emori et al., 1997).
Ayliffe and Babb (1995) reported that the process of surveillance must incorporate four key stages: data must be collected, recorded, analyzed, interpreted in the light of local circumstances, and finally presented to those who are in a position to take the necessary action.

Goals of surveillance are (Emori et al., 1997):

a- To define endemic (background) rates of infectious and noninfectious adverse events.

b- To identify increase in infection rates above the endemic level.

c- To identify specific risks for nosocomial infection for patients undergoing routine hospital care or procedures.

d- To inform hospital personnel of the risks of the care or procedures they provide to patients undergoing routine hospital care or procedures.

e- To evaluate the utility and efficiency of control measures.

II. Investigation and management of an outbreak:

A major incident or outbreak is not dependant so much on the numbers of people affected but rather on the nature of the infectious agent, the pathogenicity and the transmissibility of the microorganism (Doebelling, 1993)

It is the responsibility of the infection control committee, to draw up detailed plans appropriate the local situations and particular units for management of the incidents and outbreak in hospital (Glenister et al., 1992).
Investigation of an outbreak requires the following: (Zaza and Jarvis, 1996):

1-Confirm the existence of an outbreak
2-Verify the diagnosis
3-Create a case definition
4-Identify and count cases or exposures
5-Develop a hypothesis (e.g. mode of spread, reservoir)
6-Develop a line-listing
7-Tabulate and set the data in terms of time, place and person
8-Take immediate control measures
9-Communicate information to relevant personnel
10-Screening of personnel and environment as indicated
11-Write a cohort report (preliminary & final)
12-Summarize investigations and recommendations to the appropriate authorities.

III-Isolation measures:

Isolation precautions are designed to prevent transmission of microorganisms in hospitals (Beekman and Henderson, 1997).

Isolation is separation of patient and her or his care from other people. Patients may be at risk of infection, particularly if they have a decreased resistance and patients may also be a source of infection to others.

Microorganisms are transmitted in hospitals by several routes, and the same microorganisms may be transmitted by more than one route (Garner, 1996).
The knowledge of the routes of transmission of infection has been used to develop policies and procedures on isolation of patients with communicable disease or epidemiologically important microorganisms (Beekman and Henderson, 1997).

There are main five routes of transmission: contact, droplet, airborne, common vehicle, and vectorborne:

1-Contact transmission, the most important and frequent mode of transmission of nosocomial infections and divided into two subgroups:
   a) Direct-contact transmission involves a direct body surface-to-body surface contact and physical transfer of microorganisms between a susceptible host and an infected or colonized person (Patterson, 1996).
   b) Indirect-contact transmission involves contact of a susceptible host with a contaminated intermediate object, usually inanimate, such as contaminated instruments, needles, or dressings (Patterson, 1996).

2-Droplet transmission occurs when droplets containing the microorganism generated from the infected person are propelled a short distance through the air and deposited on the host’s conjunctivae, nasal mucosa or mouth (Garner, 1996).

3-Airborne transmission occurs by dissemination of either (a) airborne droplet nuclei (small particle residue (5um or smaller in size) of evaporated droplets containing microorganisms that remain suspended in the air for long periods of time), (b), or dust particles containing the infectious agent (Bennson, 1995).

4-Common vehicle transmission applies to microorganisms transmitted by contaminated items such as food, water, medications, devices and equipment (Bennson, 1995).
5-Vectorborne transmission occurs when vectors transmit microorganisms (Goldman et al., 1992).

* Types of isolation:

a- Barrier nursing: is a term used to describe the attempts to isolate a patient when strict physical segregation is not possible. The patients is nursed in the same multi bedded ward as other patients and is exposed to an interchange of air-borne microorganisms with them, but is protected by the practice of strict aseptic precautions against the transmission of infection from contact with the hands or clothing of attendant staff or with contaminated equipments. All attendants must wear clean gowns, must wash their hands before and after visiting the patient. All articles including eating utensils are kept for the sole use of the patient and his excreta are disinfected. After discharge all disposable material should be destroyed and the clothing, bedding, etc., used by the patient, disinfected by special wash.

b- Cubicle or single bedded room isolation: by which the patient is nursed by himself in a room separated by a door and corridor from other patients rooms, confers a substantial measure of protection against air-borne cross-infection. The protection is greatest if a system of unidirectional exhaust ventilation with clean or filtered air is used. Comparative studies of cross infection with heterologous types of streptococcus pyogens and Corynebacterium diphtheria wards have shown that the adoption of strict barrier nursing techniques in multi beeded wards brought about only slight or moderate reductions in the cross-infection rate, whilst cubicle isolation plus aseptic nursing virtually eliminated cross-infection. Strict isolation in plastic tent provides a germ free environment used mainly for susceptible patients (Rutala, 1993).
* **Standard precautions:**

Standard precautions apply to (1) blood; (2) all body fluids, secretions, and excretions except sweat regardless of whether or not they contain visible blood; (3) non intact skin; and (4) mucous membranes (Garner and HICPAC, 1996).

**Standard precautions include:**

a- Hand washing.
b- Gloving.
c- Mask, eye protection, and face shield.
d- Gown.
e- Patient-care equipment.
f- Environmental control.
g- Linen.
h- Occupational health and blood-borne pathogens.
i- Patients placement.

a- **Hand Washing:**

Hand washing is the single most important procedure for preventing nosocomial infection (Rotter; 1997).

The hands of health care workers are the main vehicles of multidrug resistant bacteria and nosocomial infection in hospital wards (Fridkin, 1997).

Hand washing with plain soaps and detergent is effective in removing many transient microbial flora (Garner and Favero, 1986). Resident microorganisms in the deep layers may not be removed by hand washing with soap and detergents, but usually can be killed or inhibited.
by hand washing with products that contain antimicrobial ingredients (e.g. N-propanol, Isopropanol and Ethanol) (Block, 1991).

1-Indications of hand washing:

a-in the absence of true emergency, personnel should always wash their hands:

- Before performing invasive procedures and before taking care of particularly susceptible patients such as those who are severely immunocompromised and newborns (Larson and Kretzer 1995). Also before and after touching wounds, whether surgical traumatic or associated with an invasive device (Ehrenkranz, 1992).

- After contact with mucous membranes, blood or body fluids, secretions or excretions (Larson et al., 1995). Also after taking care of an infected patient or one who is likely to be colonized with microorganisms of special clinical significance, for example, multiply resistant bacteria (Larson, 1993). And after touching inanimate sources that are likely to be contaminated with virulent microorganisms, for example, urine-measuring devices or secretion collection apparatuses (Larson, 1993).

- Between contacts with different patients in high risk units (Ehrenkranz, 1992).

b-Most routine hospital activities, involving direct patient contact as taking a blood pressure, or indirect patient contact such as handing a patient medications, do not require hand washing (Garner and HICP, 1996).
2-Hand washing Technique

For routine hand washing, a vigorous rubbing together of all surfaces of lathered hands for at least 10 seconds, followed by thorough rinsing under a stream of water (Larson and Kretzer 1995).

The diagram on the next page showing the best method for hand washing.

3-Hand washing with plain soap or antimicrobial containing products:

Plain soap should be used for hand washing unless otherwise indicated (Rotter, 1997).

If bar soap is used, it should be kept in racks that allow drainage of water. If liquid soap is used, the dispenser should be replaced or cleaned and filled with fresh product when empty; liquids shouldn't be added to a partially full dispenser (Larson, 1993).

Conventional handwashing takes only about 10s, the entire procedure of leaving the room, moving to the sink, adjusting the tap, drying the hands, and returning to the patient takes more than a minute (Vossand and Widmer, 1997).

Handrubbing with an alcohol solution is significantly more efficient in reducing hand contamination than handwashing with antiseptic soap (Girou et al., 2002).

Antimicrobial hand-washing products should be used for hand washing. Between patients in high-risk units, and before personnel take care of severely immuno-compromised patients (Rotter, 1997).
1- Palm to palm
2- Right palm over left dorsum and left palm over right dorsum

3- Palm to palm, fingers interlaced
4- Backs of fingers to opposing palms with finger interlocked.

5- Rotational rubbing of right thumb clasped in left palm and Vice versa
6- Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa

7- The wrists are similarly rubbed, washed and dried.

Fig. (1): Technique of hand washing
b-Gloves:

Disposable gloves (clean, non sterile gloves are adequate should be worn for direct contact with blood body fluids, secretions, excretions and contaminated items (Patterson et al., 1991).

Gloves should be changed between tasks and procedures on the same patient to ensure that bacteria from an infected or colonized site on the patient are not transferred to a susceptible site such as a wound or urine catheter. Washing gloves between patents is not recommended as the gloves may be damaged by the soap solution and if punctured unknowingly, may cause body fluid to remain in direct contract with skin for prolonged periods (Oslen et al., 1993).

Gloves should be discarded before leaving the room or before going to another patient and wash hands immediately as hands are easily contaminated during the removal of gloves (Oslen et al., 1993).

Kim et al., 2003 found that, glove use increases compliance with hand disinfection, because workers don’t appropriately comply with disinfection guidelines.

c- Mask, eye protection, Face shield:

Microorganisms can pass through mucous membranes to cause infection, healthcare workers have acquired HIV as a result of blood splashing into the face. A mask that covers both the nose and the mouth, and goggles or face shield are worn by hospital personnel during procedures and patient care activities that are likely to generate splashes or sprays of blood, body fluids, secretions, or excretions to provide protection of the mucous membranes of the eyes, nose, and mouth from
d-Gown:

Gowns are worn to prevent contamination of clothing and to protect the skin of personnel from blood and body fluid exposures also to reduce the risk of cross-infection of microorganisms to other patients on the clothing (Wong et al., 1991).

Select a gown (a clean, non sterile is adequate) that is appropriate for the activity and amount of fluid likely to be encountered. Soiled gowns should be removed as promptly as possible and wash hands directly after removal (Department of Labor, Occupational Health & Safety 1991).

e-Patient-care equipment:

Patient care equipment soiled with blood, body fluids secretions and excretions in a manner that prevents skin and mucous membrane exposures. Also avoid contamination of clothing and transfer of microorganisms to other patients and environments (Rutula, 1993).

Contaminated reusable critical medical devices or patient care equipment (i.e., equipment that enters normally sterile tissue or through which blood flows) or semicritical medical devices or patient-care equipment (i.e, equipment that touches mucous membranes) are sterilized or disinfected (reprocessed) after use to reduce the risk of transmission of microorganisms to other patients. Non-critical equipment (i.e, equipment that touches intact skin) contaminated with blood, body fluids or
Secretions is cleansed and disinfected after use, according to the hospital policy (Favero and Bond, 1991).

f- Environmental control:

Ensure that the hospital has adequate procedures for the routine care, cleaning, and disinfection of environmental surfaces, beds, bedside equipment and other frequently touched surfaces. This is indicated for certain pathogens, especially enterococci, which can survive in the inanimate environment for prolonged periods of time (HICPAC, 1995).

g- Linen:

Although soiled linen may be contaminated with pathogenic microorganisms the risk of disease transmission negligible. This is achieved if linen is handled, transported, and laundered in a manner that avoids transfer of microorganisms to patients, personnel and environments (Joint Committee on Healthcare Laundry Guidelines, 1994).

h- Occupational health and blood-borne pathogens:

Sharp instruments frequently cause injury to health care workers and are a major cause of transmission of blood-borne viruses (Advisory Committee on Dangerous Pathogens, 1995).

Safe use, handling and disposal of sharps:

Sharps are any items that can cause laceration or puncture wounds. Examples include discarded hypodermic needles, instruments used in invasive procedures (e.g., blood sampling and surgery); emergency services cutting equipment.
Methods of safe disposal of sharps (*Collins and Kennedy, 1993; Gwyther, 1990*).

1-It is the personal responsibility of the person using a sharp to dispose of it safely after use.
2-Needles and syringes should be discarded as a single unit, where this is possible, into a designated sharp box.
3-Glass slides, glass drug ampoules, razors and IV cannulae must be discarded into a sharp box.
4-The attached sharps from IV blood/solution administration sets must be cut off with scissors and discarded into sharps box.
5-When syringes containing arterial blood are to be sent to the laboratory, needles must be removed and the nozzles of the syringes sealed by means of a rubber cap.
6-Health care workers must not reuse the barrel (needle holder) of vacuum blood collection systems where there is visible blood contamination or where blood has been taken from a patient who is known or suspected to be infected with blood-born virus.
7-When an injury occurs with a contaminated sharp, bleeding should be encouraged and the site should be washed under running water. The injured member of staff should immediately report the incident to their line manager who should then refer them to the Occupational Health Department or to the Local Accident and Emergency department according to local policy.

(i) Patient placement:

Appropriate patient placement is a significant component of isolation precautions. A private room is important to prevent direct-or indirect-contact transmission when the source patient has poor hygienic habits or contaminates the environment (*Garner, 1996*).
IV- Cleaning, disinfection and sterilization:

The decision to clean, disinfect or sterilize depends on the risk of the equipment transmitting infection.

As a general rule, methods of sterilization or disinfection employing heat, such as autoclaves and bed pan washers, are more reliable than chemicals and should be used whenever feasible.

V- Employee health service:

Health care workers are exposed to the most diverse group of occupational hazards, including biologic, chemical, and physical agents as well as injury and psychological stress (Lewy, 1987).

The American Hospital Association and the American Medical Association recognized the importance of the employee health service in 1957 through the development of a joint committee on Health Programs for hospital Personnel (Lewy, 1987).

This joint report outlined essential components of a comprehensive program, which are still relevant today. The important components include the following (Lewy, 1987).

a- Preplacement medical examinations.
b- Periodic health examinations
c- Routine immunizations
d- Health counseling
e- Available care for work-related injury and illness
f- Regular environmental inspections for hazards in the work place.
g- Health and safety education programs
h- Employee health and safety record-keeping systems with regular review of exposure, illness, and injury.
i- Coordinated planning with other hospital services and departments.
The employee health department plays a critical role in protecting the health and safety of workers and their patients (Department of Labor, Occupational Safety and Health, 1991).

VI-Disposal of clinical waste:
Health care facilities that generate medical, chemical, or radiological waste have a moral and legal obligation to dispose of their wastes in a manner that poses minimal potential hazard to the environment or public health (Rutala, 1997).

Hospital waste refers to all waste, biologic or non-biologic, which is discarded and not intended for further use.

Medical waste: refers to materials generated as a result of patient diagnosis, immunization or treatment such as soiled dressings or I.V tubing (Rutala et al., 1992).

Infectious waste refers to the portion of medical waste that could potentially transmit an infectious disease (Rutala and Weber, 1991).

For a waste to be infectious it must contain pathogens with sufficient virulence and quantity so that exposure to the waste by susceptible host could result in an infectious disease (Garner and Favero, 1986).

Treatment of infectious waste: by U.S. hospitals is most commonly accomplished by incineration (range 64% to 93%, depending on type of waste). About one-third of U.S. hospitals steam sterilize their microbiologic waste, and about one-fourth pour liquid blood done the drain connected to a sanitary sewer (Rutala, 1997).
The presence of preoperative antibodies to gram negative lipopolysaccharides has a protective effect against post operative infections and pyrexia in patients undergoing surgical procedures, so in future immunization may be considered an adjunct or alternative to prophylactic antibiotics.

*The following procedures must be done:

Tests on autoclaves and hot-air oven should be made as a routine, it is important that any insects do not invade dressing packs or equipment or clean laundry. The arrangements made for cleaning and preparing trolleys should be checked. Bacteria are liable to grow in ordinary corks and these should never be used (Cruse and Foord, 1980).

*Preparation of patients:

Beds, blankets and other potentially contaminated materials from the wards should not be allowed into the theater. The patient should be covered with freshly laundered gown, sheet or cotton blanket before entering the theater. The solution used to disinfect the skin should be suitable for the purpose and used in the correct strength (CDC, 1985).

*Surgical procedures:

The surgical team should shower up adequately, preferably using a disinfectant. Contrary to what might be expected, there are objections to taking a shower immediately before duty, as most people shed more bacteria into the air after a shower than before it. This effect lasts for an hour. The practice of putting on freshly laundered clothes before putting on a gown should be encouraged. Although carriers of S.aureus still disperse organisms when dressed in this way, the number of organisms liberated are less than when a gown is worn over everyday clothes.
MATERIAL AND METHODS
Material AND METHODS

This study comprised collection of microbiological samples from environment, patients and working staff in intensive care unit in Benha university hospital, during the period from April 2001 to March 2002.

I-Environmental samples:

1-Air samples:

Air samples were taken from twenty location in the ICU by using blood agar exposure plates and standard RCS air samplers at the same time.

a) Blood agar exposure plates:

These plates were put opened for 2 hours then collected, incubated at 37°C for 48h and then colony counts were done to estimate quantitatively microbiological air contamination.

b) Standard RCS Air sampler (Boiotest landsteiners Tr. 5D- 63303 Dreieich Germany):

Standard RCS air sampler is a convenient instrument for measuring the concentration of air born microorganisms (number of microbial colony-forming units “CFUs” in room air). It helps in periodic assessment of air quality of microbial levels.

Principle of the technique:

The function of air sampler is to deposit air borne microorganisms quantitatively onto a culture medium.

The air under examination is drawn into the sampler from a distance of at least 40cm by means of an impeller.
- The sampling time ranges from 30 seconds to 8 minutes.
- The sampling air capacity ranges from 20-30 liters with a separation volume of 40 liters per minutes.
- Media strips containing agar are used to line the sampler drum.
- Each strip is divided into $34 \times 1 \text{ cm}^2$ section.

The air flow enters the impeller drum concentrically and in a conical form, is set in rotation, and the particles confined in the air are impacted by centrifugal form onto an agar medium. The air then leaves the drum in a spiral form around the outside of the cone of air entering the sampler. The sampling time was for 2 minutes. After sampling, the agar strip is incubated for 24h and the colonies are counted.

Microbial colonies are enumerated after incubation by visual examination directly through the sealed agar strip wrapper. The microbial count (CFUs) were calculated as follows:

$$CFU/L = \frac{\text{colonies on Agar strip}}{40 \times \text{sampling time (minutes)}}$$

Fig. (1) Air sampler RCS
2- Environmental swabs:
    Twenty swabs were taken from each of the following:
    - Laryngoscopes.
    - Ventillators.
    - Air condition grill.
    - Beds.

    Samples were inoculated into blood and MacConkey agar plates, then the plates were incubated for 24h at 37°C. The isolates of swabs and exposure plates were identified to the species level and tested for the action of disinfectants by microtitration method.

II-Workers samples:
    Fifty samples from both nasal and hand swabs were taken from I.C.U workers, their age ranged from 20 to 48 years (30 females and 20 males) to detect MRSA-carriers.

    Hand swabs: were collected from finger nails and from between the fingers before handwashing.

III-Patients samples:
    Fifty Nasal and rectal swabs were taken to detect multi-drug resistant carriers from patients in ICU (32 females and 18 males) their age ranged from 32 to 68 years.

    Nasal swabs: for detection of MRSA
    They were taken from the examined patients by a circular movement of the swabs within the distal one cm of anterior nares (five rotation on each side) according to Winkler et al., (1990).
Rectal swabs: for detection of ESBL

Patients and workers specimens were subjected to the following:
1- Isolation of the organisms by culturing on ordinary bacteriological media (blood, chocolate and MacConkey agar) and incubating aerobically at 37°C for 48 hours.
2- Gram stained smear for the isolated pathogen.
3- Biochemical reaction of the isolates.
   For gram negative bacilli:
   a- Oxidase test.   b- API 20 E.
   For gram positive cocci:
   a- Catalase test: to differentiate Staphylococci (+ve) from Streptococci (-ve).
   b- Coagulase test: to identify S. aureus
   c- DNase test: to identify S. aureus
   d- API staph.
4- Antimicrobial susceptibility test for the isolated pathogen.
   - For gram negative bacilli for detection of ESBL.
   - For gram positive cocci for detection of MRSA.
5- The identified isolates were tested for the action of disinfectants by microtitration and tube neutralization methods.

* For gram negative bacilli:
   a- Oxidase test:
       For identification of Pseudomonas.
b-API 20 E supplied by (BioMérieux):

Identification system for enterobacteriaceae and other gram-negative rods.

**Principle:**

The API 20 E strip consists of 20 microtubes containing dehydrated substrate. These tests are inoculated with a bacterial suspension that reconstitutes the media during incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents.

The reactions are read according to the reading table. The identification is obtained by referring to the Analytical Profile Index.

**Procedure:**

*Preparation of specimens:

The microorganisms was first isolated from MacConkey agar. Single separate colony was chosen to make the test.

* Preparation of the strip:

The incubation box (tray and lid) was prepared and about 5ml of distilled water was distributed into the honey combed wells of the tray to create a humid atmosphere. The strip was removed from its packaging. The strip was placed in the incubation box.

* Preparation of the inoculum:

- A single well-isolated colony was removed from an isolation plate.
- The colony was emulsified in the suspension medium to achieve a homogenous bacterial suspension (0.5 McFerland).
* Inoculation of the strip:
- Both tube and cupule of the tests (CIT), (VP) and (GEL) were filled with the bacterial suspension.
- The tube was field (and not the cupule) of the other tests.
- Anaerobiosis was created in the tests ADH, LDC, ODC, H2S and URE by overlaying with mineral oil.
- The incubation box was closed.
- The strip was incubated at 37°C for 24 hours.

* Reading the strip:
After 24 hours at 37°C, the strip was read by referring to the reading table.
- All spontaneous reactions were recorded on the result sheet and follow manufactures instruction.

* Identification:
Identification was obtained by Analytical Profile Index.

Fig. (2): API 20 E strip

* For gram positive cocci:
a-Catalase test:
For differentiation between Staphylococci and Sterptococci;

Principle:
Principle:

Staphylococci produce catalase enzyme which breaks H₂O₂ into O₂ + H₂O resulting in rapid production of bubbles.

b-Coagulase test:

This test was done to differentiate between coagulase positive S. aureus and coagulase -ve S. aureus (S. epidermidis and S. saprophyticus).

i) Slide coagulase test:

This rapid test detects bound coagulase (clumping factor). It is the main method used to identify S. aureus in clinical laboratory (Collee et al., 1989).

ii) Tube coagulase test:

This test for S. aureus was performed with citrated plasma as for the slide test.

c-Deoxy ribonuclease (Dnase) test:

Most strains of S. aureus hydrolyse DNA and give positive reaction by using DNA agar (Oxoid) according to the method modified from Jeffries (1961).

Dnase positive are those spot cultures that are surrounded by clear, uncloudy zones comparable in width to the zone around the control DNase-positive culture.

d-API STAPH: (supplied by BioMérieux)

Identification system for Staphylococci and Micrococci.
Principle:

API STAPH consists of a strip containing dehydrated test substrates in individual microtubes. The tests are reconstituted by adding to each tube an aliquot of API STAPH Medium that has been inoculated with the strain to be studied.

* Preparation of the strip:

- The incubation box (tray and lid) was prepared by distributing about 5ml of distilled water into the honey-combed wells of the tray to create a humid atmosphere.
- The strip was removed from its individual packaging.
- The strip was placed in the incubation box.

* Preparation of the inoculum:

- The strain was confirmed that it belong to the Micrococcaceae family (morphology Gram stain, catalase etc) and also, it was confirmed check that the culture is pure.
- The ampoule of API STAPH Medium was opened.
- A homogeneous bacterial suspension with a turbidity equivalent to 0.5 McFerland was prepared.

* Inoculation of the strip:

- The microtubes were filled with the inoculated API STAPH Medium. Only the tube portion of the microtubes was filled, not the cupules. To avoid the formation of bubbles at the base of the tubes, the strip was tilted slightly forwards and the tip was placed of the pipette against the side of the cupule
- Anaerobiosis was ensured in the ADH and URE tests by filling the cupules with mineral oil to form convex meniscus.
- The incubation box was closed and incubated at 35-37°C for 18-24 hours.

*Reading the strip:*
- The reaction was developed by adding 1 drop of each of the following reagents and then all the reactions were read by referring to the Reading Table.
- VP test: VP1 and VP2 reagents. A violet-pink color after 10 minutes indicates a positive reaction. A pale pink or light pink color obtained after 10 minutes should be considered negative.
- NIT test: NIT 1 and NIT2 reagents. A red color after 10 minutes indicates a positive reaction.
- PAL test: ZYMA and ZYMB reagents. A violet color after 10 minutes indicates a positive reaction.
- The results were recorded on the result sheet.

*Identification:*
Identification was obtained by Analytical Profile Index.

![Fig. (3): API Staph strip](image-url)

**Antibiotic Susceptibility Testing:**
All isolates were tested for their in-vitro susceptibility to various antibiotics (Oxoid) by agar disc diffusion methods *(Bauer et al., 1966).*

**Procedure:**
1- Several colonies of similar appearance of the tested organism were emulsified in a small volume of sterile nutrient broth.
2- The turbidity of the suspension was adjusted to 0.5 McFerland turbidity standard.

3- A loopful, of the tested organism suspension was applied to plate of Mueller Hinton agar, a sterile dry non-absorbant swab was used to spread the inoculum across the plate.

4- The inoculum was allowed to dry for a few minutes with the dish lid in place.

5- By using sterile forceps, the antimicrobial discs were placed (previously warmed to room temperature) on the surface of sensitivity plate. Each disc was pressed down on the medium and wasn’t moved once in place.

For gram positive organisms (Staphylococci) the following discs were used: Imipenem, Amoxycilin + Clavulnic acid, Rifampicin, Cephalexin, Gentamycin, Ceftazidime, Fusidic acid, Ampicillin+ Sulbactam, Kanamycin, Cephradine, Amoxicillin, Vancomycin, Tobramycin, Penicillin, Methicillin.

For gram negative bacilli the following discs were used: Imipenem, Amoxycilin + Clavulnic acid, Rifampicin, Cephalexin, Gentamycin, Ceftazidime, Ampicilllin+Sulbactam, Kanamycin, Cephradine, Amoxicillin, Tobramycin, Penicillin.

6- The plate, was incubated aerobically at 37°C overnight (within 30 minutes of applying the discs).

The radius of the inhibition zone from the edge of the disc to the edge of the zone was measured and compared with reference zones.
*Interpretation of results:*

- **Sensitive:**
  Equal or wider than accepted zone in anti-microbial susceptibility chart.

- **Resistant:**
  No zone or zone smaller than accepted zone diameter in anti-microbial susceptibility chart (national committee for clinical laboratory standards).

**Evaluation of different disinfectants against testing isolates to different types of disinfectants:**

**The used disinfectants were:**

1- Alkanol (iso propanol + H₂O₂).
2- Polyseptol (chloroxylenol + dichloroxylenol + cetrimide).
3- Cidex (Glutaraldehyde 2%).
4- Clorox (Sodium hypochlorite).
5- Hydrocil (H₂O₂)
6- MAda Cide-1 (Dimethyl benzyl ammonium chloride + dimethyl ethylbenzyl ammonium chloride).

All the above disinfectants were subjected for:

**I-Microtitration methods:**

For determination of the bactericidal and fungicidal activities using the method described by Russell et al., (1983).
**Principle:**

The disinfectant was mixed with nutrient broth in serially decreasing concentrations, the microtitration plate was inoculated with the bacterial suspension to be tested and after a suitable period of incubation, the lowest concentration which inhibits the growth of the organism was considered as MIC value of the disinfectant used.

*Material preparation:*

1- Preparation of double strength broth:

Sixteen grams of nutrient broth powder were dissolved in 1000 ml D.W. then medium was sterilized in the autoclave. It was left to cool to room temperature, it was kept in the refrigerator at 4°C.

2- Preparation of bacterial and fungal suspensions:

Pure colonies of the tested microorganism were collected to prepare a homogenous suspension in nutrient broth to a turbidity of 0.5 McFerland.

*Test procedure:*

1- Fifty microliters of the bacterial suspension were put in the wells of microtitration plate at least in 7 wells.

2- Fifty microliters of the tested disinfectant were added to the first well of microtitration plate, then serial dilution was made in the next wells till the dilution of 1/64 in the last well.
3- The organism-broth- disinfectant mixture were incubated at 37°C for 24 h. All the disinfectants and antiseptics were tested simultaneously with the same bacterial suspension at the same environmental conditions.

4- The plates were examined for the presence of growth or no, the growth is observed by the presence of turbidity.

* **Interpretation of the results:**

The lowest concentration of the disinfectant which inhibit the growth of the organism was the minimum inhibitory concentration of the disinfectant.

**II- Bactericidal activity of different type of disinfectants using the British/European standard test method: BSEN 1276: 1997.**

- **Test method:**
Tube neutralization method

- **Organism tested:**
S. aureus, MRSA, Klebsiella spp. Pseudomonas aeruginosa and Candida spp. (all are hospital strains).

- **Temp.:**
  20 ± 1°C

- **Media used:**
Sterile broth, blood agar, MacConkey agar and Sabaroud agar media
• **Incubation:**

1) For 2, 5 and 10 minutes at 37°C & 28°C.

2) For overnight at 37°C for bacteria and at 28°C for fungus.

• **Test procedure:**

A 0.5 MacFerland of bacterial and candida suspensions was prepared using sterile broth.

A working solution of the disinfectants was prepared by adding tap water (except cidex).

Addition of \( \frac{1}{2} \) ml of standard suspension of organism was added to 2ml of disinfectants and left in the incubator for a contact time \( \frac{2}{5} \) and \( \frac{10}{30} \) minutes subcultures was done on solid media as blood for Staphylococci and MacConkey for Pseudomonas, Klebsiella and Sabaroud for Candida.

All were incubated for overnight.

**Laboratory testing of the Mycobacterium tuberculosis to different types of disinfectants:** *(Best et al., 1990)*.

**Procedure:**

1- M. tuberculosis bacilli (not nosocomial and were two cases and done as a trial) were emulsified in sterile water to the turbidity of 0.5 MacFerland.

2- One volume of suspension was added to two volumes of the tested disinfectant. This mixture is kept in incubator at 37°C for one hour.

3- This mixture was cultured on Lowenstein Jensen media and kept in incubator at 37°C for 6 to 8 weeks.
4- The cultured tubes were examined every 3 days for any growth.

**III-In-use test is done as a reference test for different disinfectants:**

The best valid test of a bactericidal product is its evaluation in the field under actual conditions of use to assess its performance in the prevention of contamination (*Kelsey and Maurer, 1966*).

*Test principle:*

Using the method of *Kelsey and Maurer (1966)*:

An effective preparation for surface or instrument disinfection should not retain surviving bacteria after use, the solution should sufficiently bactericidal, so that, despite the load by dirt, blood or serum, it continues to kill the germs within a short time. It is helpful in monitoring the disinfection practice in a hospital. A sample was taken from bucket contents after cleaning and from containers for contaminated instruments.

*Material preparation:*

1- Preparation of nutrient broth.

2- Nutrient agar plates.

3- Sterile pasteur pippette.

*The test steps:*

1- One ml of the disinfectant in use was added to nine ml of the nutrient broth to make one in ten dilution of disinfectant in nutrient broth.
2- Ten drops of this mixture was placed on two nutrient agar plates using the sterile pasteur pipette.

3- One plate was incubated at 37°C for 3 days and the other plate at room temperature for 7 days.

*Interpretation of the results:
- Growth of more than 5 colonies on either plate after incubation indicate failure of the disinfectant in use.
- Growth of less than 5 colonies was accepted.
RESULTS
RESULTS

The results of this study are presented in the following tables and figures.

Microorganisms isolated from studied groups:

Frequency and percentage of microorganisms isolated from air taken from twenty location in ICU are illustrated in table (1):

Table (1): Frequency and percent of microorganisms isolated from air

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>18</td>
<td>32.7%</td>
</tr>
<tr>
<td>Coagulase negative staph</td>
<td>15</td>
<td>27.3%</td>
</tr>
<tr>
<td>Gram negative bacilli</td>
<td>12</td>
<td>21.8%</td>
</tr>
<tr>
<td>Candida</td>
<td>10</td>
<td>18.2%</td>
</tr>
<tr>
<td>Total no. of organisms</td>
<td>55</td>
<td>100%</td>
</tr>
</tbody>
</table>
The comparison between colony counts taken from twenty locations in ICU which were obtained by strips and exposure plates, is illustrated in table (2), Fig. (1, 2).

Table (2): comparison between colony counts obtained by strips and exposure plates.

<table>
<thead>
<tr>
<th>Location</th>
<th>Strips (CFU)</th>
<th>Exposure plates (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>190</td>
<td>180</td>
</tr>
<tr>
<td>L2</td>
<td>236</td>
<td>145</td>
</tr>
<tr>
<td>L3</td>
<td>635</td>
<td>600</td>
</tr>
<tr>
<td>L4</td>
<td>850</td>
<td>400</td>
</tr>
<tr>
<td>L5</td>
<td>680</td>
<td>240</td>
</tr>
<tr>
<td>L6</td>
<td>238</td>
<td>200</td>
</tr>
<tr>
<td>L7</td>
<td>374</td>
<td>260</td>
</tr>
<tr>
<td>L8</td>
<td>340</td>
<td>160</td>
</tr>
<tr>
<td>L9</td>
<td>306</td>
<td>360</td>
</tr>
<tr>
<td>L10</td>
<td>374</td>
<td>400</td>
</tr>
<tr>
<td>L11</td>
<td>442</td>
<td>400</td>
</tr>
<tr>
<td>L12</td>
<td>516</td>
<td>420</td>
</tr>
<tr>
<td>L13</td>
<td>221</td>
<td>301</td>
</tr>
<tr>
<td>L14</td>
<td>621</td>
<td>350</td>
</tr>
<tr>
<td>L15</td>
<td>200</td>
<td>160</td>
</tr>
<tr>
<td>L16</td>
<td>410</td>
<td>390</td>
</tr>
<tr>
<td>L17</td>
<td>372</td>
<td>400</td>
</tr>
<tr>
<td>L18</td>
<td>522</td>
<td>371</td>
</tr>
<tr>
<td>L19</td>
<td>235</td>
<td>198</td>
</tr>
<tr>
<td>L20</td>
<td>400</td>
<td>280</td>
</tr>
</tbody>
</table>

L = Location       CFU = colony forming unit

Colony count obtained by strips was higher in (80%) of samples to that obtained by exposure plates.
Figure (2): Comparison between C.C. obtained by RCS air sampler and blood agar exposure plate.

C.C.: Colony count
(L=Location)

Blood agar exposure
RCS AIR Sampler
Fig. (3): Air samples taken from different location in the ICU by exposure plates
Fig. (4): Air samples taken from different location in the ICU by standard RCS air sampler
Results

Frequency and percentage of microorganisms isolated from environmental swabs (Laryngoscopes ventilators, air condition grills and beds) are illustrated in table (3).

Table (3): Frequency and percent of microorganisms isolated from environmental swabs.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>22</td>
<td>44 %</td>
</tr>
<tr>
<td>Coagulase negative staph</td>
<td>10</td>
<td>20 %</td>
</tr>
<tr>
<td>Gram negative bacilli</td>
<td>18</td>
<td>36 %</td>
</tr>
<tr>
<td>Total no. of organisms</td>
<td>50</td>
<td>100%</td>
</tr>
</tbody>
</table>

Microorganisms isolated from workers samples

a) Hand samples

Frequency and percentage of bacteria in fifty samples taken from workers hands are shown in Table (4), Fig (5).

S. epidermidis was the commonest isolate (40%) followed by S. aureus (24%), S. saprophyticus (20%) and S. haemolyticus (16%).

Table (4): Frequency and percent of bacteria in hand swabs from workers

<table>
<thead>
<tr>
<th>Organism</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>20</td>
</tr>
<tr>
<td>S. aureus</td>
<td>12</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>10</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>8</td>
</tr>
<tr>
<td>Total no. of organisms</td>
<td>50</td>
</tr>
</tbody>
</table>
Figure (5): Percent of bacteria in hand swabs from workers.
b) Nasal samples

The frequency of organisms in fifty samples taken from workers nose are illustrated in Table (5), Fig. (6). S. aureus was 50% and also S. epidermidis was 50%.

Table (5): Frequency and percent of bacteria in nasal swabs from workers.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>25</td>
</tr>
<tr>
<td>S. aureus</td>
<td>25</td>
</tr>
<tr>
<td>Total no. of organisms</td>
<td>50</td>
</tr>
</tbody>
</table>

Fig. (6): Percent of bacteria in nasal swabs from workers
Fig. (7): API reaction profile of different isolated staphylococci
Microorganisms Isolated from patients samples

a) Nasal samples

Frequency of bacteria in fifty nasal samples taken from patients are illustrated in Table (6) Fig. (8), the most common isolate was S. aureus (40%). Gram-positive cocci represented (66%) of isolates while gram-negative bacilli represented (34%).

Table (6): Frequency and percent of bacteria in nasal swabs from patients.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>S. aureus</td>
<td>20</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>3</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>4</td>
</tr>
<tr>
<td>E coli</td>
<td>5</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>5</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>1</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>7</td>
</tr>
<tr>
<td>Pseud. aeruginosa</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>3</td>
</tr>
<tr>
<td>Total no. of organisms</td>
<td>50</td>
</tr>
</tbody>
</table>

Fig. (8): Percent of bacteria in nasal swabs from patients
b) Rectal samples

The frequency and percent of bacteria in fifty rectal samples taken from patients are illustrated in Table (7), Fig. (9).

The most common isolate was E. coli (38%) of the all isolated organisms.

Table (7): Frequency and percent of bacteria in rectal swabs from patients.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Citrobacter koseri</td>
<td>5</td>
</tr>
<tr>
<td>A. hydrophili</td>
<td>4</td>
</tr>
<tr>
<td>C. freundii</td>
<td>4</td>
</tr>
<tr>
<td>E. coli</td>
<td>19</td>
</tr>
<tr>
<td>K. ornithinolyticus</td>
<td>4</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>5</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td>5</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>4</td>
</tr>
<tr>
<td>Total no. of organisms</td>
<td>50</td>
</tr>
</tbody>
</table>

Fig. (9): Percent of bacteria in rectal swabs from patients.
Fig. (10): API reaction profile of different isolated Enterobacteriaceae
Results of antimicrobial susceptibility of different organisms isolated

Workers samples

a) Hand samples

The frequency and percent of MRSA which were detected from S. aureus isolates from workers hands are illustrated in Table (8). The percentage of MRSA was 8.3%.

Table (8): Frequency and percent of MRSA isolated from workers hands.

<table>
<thead>
<tr>
<th></th>
<th>Methicillin</th>
<th>Vancomycin</th>
<th>Fnsidic acid</th>
<th>Rifampicin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Sensitive</td>
<td>11</td>
<td>(91.7%)</td>
<td>12</td>
<td>(100%)</td>
</tr>
<tr>
<td>Resistant</td>
<td>1</td>
<td>(8.3%)</td>
<td>0</td>
<td>(0%)</td>
</tr>
</tbody>
</table>
b) Nasal samples

Antibiotic sensitivity and resistance of the two organisms isolated from workers nose towards different antibiotics are illustrated in Table (9).

Table (9): Antimicrobial susceptibility of bacteria isolated from nasal swabs of workers.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>S. aureus</th>
<th></th>
<th></th>
<th>S. epidermidis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
<td>Resistant</td>
<td>Sensitive</td>
<td>Resistant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Imipenem</td>
<td>20</td>
<td>(80%)</td>
<td>5</td>
<td>(20%)</td>
<td>17</td>
</tr>
<tr>
<td>Amoxycillin+ clavulinic acid</td>
<td>20</td>
<td>(80%)</td>
<td>5</td>
<td>(20%)</td>
<td>17</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>23</td>
<td>(92%)</td>
<td>2</td>
<td>(8%)</td>
<td>22</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>17</td>
<td>(68%)</td>
<td>8</td>
<td>(32%)</td>
<td>15</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>17</td>
<td>(68%)</td>
<td>8</td>
<td>(32%)</td>
<td>22</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>20</td>
<td>(80%)</td>
<td>5</td>
<td>(20%)</td>
<td>22</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>22</td>
<td>(88%)</td>
<td>3</td>
<td>(12%)</td>
<td>25</td>
</tr>
<tr>
<td>Ampicillin + Sulbactam</td>
<td>15</td>
<td>(60%)</td>
<td>10</td>
<td>(40%)</td>
<td>17</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>8</td>
<td>(32%)</td>
<td>17</td>
<td>(68%)</td>
<td>12</td>
</tr>
<tr>
<td>Cephadine</td>
<td>15</td>
<td>(60%)</td>
<td>10</td>
<td>(40%)</td>
<td>20</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>12</td>
<td>(48%)</td>
<td>13</td>
<td>(52%)</td>
<td>20</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>25</td>
<td>(100%)</td>
<td>0</td>
<td>(0%)</td>
<td>25</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>17</td>
<td>(68%)</td>
<td>8</td>
<td>(32%)</td>
<td>20</td>
</tr>
<tr>
<td>Penicillin</td>
<td>10</td>
<td>(40%)</td>
<td>15</td>
<td>(60%)</td>
<td>5</td>
</tr>
<tr>
<td>Methicillin</td>
<td>15</td>
<td>(60%)</td>
<td>10</td>
<td>(40%)</td>
<td>22</td>
</tr>
</tbody>
</table>
MRSA strains were detected in nasal samples from workers. They represented (40%) of isolated S. aureus and it is illustrated in Table (10), Fig. (11).

**Table (10): Frequency and percent of MRSA isolated from nasal swabs of workers.**

<table>
<thead>
<tr>
<th>Response</th>
<th>Methicillin</th>
<th>Rifampicin</th>
<th>Vancomycin</th>
<th>Fusidic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.  %</td>
<td>No.  %</td>
<td>No.  %</td>
<td>No.  %</td>
</tr>
<tr>
<td>Sensitive</td>
<td>15 (60%)</td>
<td>23 (92%)</td>
<td>25 (100%)</td>
<td>22 (88%)</td>
</tr>
<tr>
<td>Resistant</td>
<td>10 (40%)</td>
<td>2 (8%)</td>
<td>0 (0%)</td>
<td>3 (12%)</td>
</tr>
</tbody>
</table>

![Graph showing the percentage of MRSA isolated in nasal samples from workers.]

**Fig. (11): Percent of MRSA isolated in nasal samples from Workers.**

**Patients samples**

a) Nasal samples

The results of antimicrobial susceptibility testing of gram-negative and gram positive isolates from nasal samples of patients are illustrated in Table (11).
MRSA was detected in (80%) of isolated S. aureus. The antimicrobial susceptibility of MRSA is illustrated in Table (12), Fig. (12).

Vancomycin was the most effective antibiotic against MRSA (100%) susceptible.

Table (12): Frequency and percent of MRSA in nasal swabs from patients.

<table>
<thead>
<tr>
<th>Response</th>
<th>Methicillin</th>
<th>Rifampicin</th>
<th>Vancomycin</th>
<th>Fusidinic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Sensitive N(%)</td>
<td>4</td>
<td>(20%)</td>
<td>18</td>
<td>(90%)</td>
</tr>
<tr>
<td>Resistant N(%)</td>
<td>16</td>
<td>(80%)</td>
<td>2</td>
<td>(10%)</td>
</tr>
</tbody>
</table>

Fig. (12) : Percent of MRSA in nasal swabs from patients.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Antimicrobial Susceptibility of Bacteria Isolated from Rectal Samples of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1 (13): Antimicrobial Susceptibility of Bacteria Isolated from Rectal Samples of Patients.
b) Rectal samples

Results of antibiotic susceptibility testing to the isolated microorganisms obtained from rectal samples of patients are illustrated in Table (13).

The percentage of ESBL in patient’s rectal samples is illustrated in table (14).

Table (14): Detection of resistant pattern of ESBL producing gram negative bacilli according to IPM susceptibility.

<table>
<thead>
<tr>
<th>Organism response</th>
<th>Ceftazidime</th>
<th>Imipenem</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-n(%)</td>
<td>7(37.1%)</td>
<td>14(73.5%)</td>
</tr>
<tr>
<td>R-n(%)</td>
<td>12(62.9%)</td>
<td>5(26.5%)</td>
</tr>
<tr>
<td>Aeromonas hydrophili</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-n(%)</td>
<td>4(100%)</td>
<td>4(100%)</td>
</tr>
<tr>
<td>R-n(%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Citobacter freundii</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-n(%)</td>
<td>4(100%)</td>
<td>4(100%)</td>
</tr>
<tr>
<td>R-n(%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-n(%)</td>
<td>3(60%)</td>
<td>5(100%)</td>
</tr>
<tr>
<td>R-n(%)</td>
<td>2(40%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Citrohacter koseri</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-n(%)</td>
<td>4(80%)</td>
<td>5(100%)</td>
</tr>
<tr>
<td>R-n(%)</td>
<td>1(20%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-n(%)</td>
<td>3(60%)</td>
<td>4(80%)</td>
</tr>
<tr>
<td>R-n(%)</td>
<td>2(40%)</td>
<td>1(20%)</td>
</tr>
<tr>
<td>Klebsiella ornithinolyticus</td>
<td>2(50%)</td>
<td>4(100%)</td>
</tr>
<tr>
<td>S-n(%)</td>
<td>2(50%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>R-n(%)</td>
<td>2(50%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-n(%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>R-n(%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

S= Sensitive  R= Resistant.

ESBL represent (62.9%) of E. coli, (50%) of K. ornithinolitica, (40%) of both “K. oxytoca, and P. mirabilis” and (20%) of C. koseri.
As shown in Table (14) imipenem was very effective in all isolated organisms.

**Laboratory trial of disinfectants**

The effectiveness of different types of disinfectants towards organisms isolated from different environmental samples is illustrated in Table (15).

**Table (15): Effectiveness of different types of disinfectants against different environmental samples.**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Alkanol</th>
<th>Polysptol</th>
<th>Clorox</th>
<th>Hydrocil</th>
<th>Mada-cide 1</th>
<th>Cidex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Laryngoscope</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ventilator</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Air condition grill</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Beds</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+Growth - No growth

The potency of different disinfectants towards organisms isolated from environmental samples were as follows:

- **In air samples:** Alkanol, Hydrocil, Cidex and Mada-cide 1 were effective in the inhibition of the growth of microorganism while polysptol was not effective.

- **In laryngoscope, Ventilator and air condition grill samples:** Alkanol, Hydrocil, clorox, Mada-cide 1 and cidex were effective in inhibition of growth of microorganisms, while polysptol was not effective.

- **In bed samples,** all used disinfectants were effective in inhibition of growth of microorganisms.

The MIC values of different disinfectants versus Staphylococci isolated from environmental samples are illustrated in Table (16), Fig. (13).
Cidex was the most potent 1/16 followed by clorox 1/8, Madacide-1 1/4 polyseptol 1/2, and lastly hydrocil and alkanol.

Table (16): Minimal inhibitory concentration (MIC) of different types of disinfectants against Staphylococci

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Disinfectant concentration</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc.</td>
<td>1/2</td>
</tr>
<tr>
<td>Alkanol</td>
<td>Solution</td>
<td>-</td>
</tr>
<tr>
<td>Polyseptol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Clorox</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydrocil</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mada-cide 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cidex</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ growth  - no growth

Fig.(13) : Minimal inhibitory concentration (MIC) of different types of disinfectants against Staphylococci
The MIC values of different disinfectants versus gram negative bacilli isolated from environmental samples are illustrated in table (17), Fig (14).

The most potent disinfectants working against gram negative bacilli were cidex and clorox 1/8 followed by alkanol 1/4, hydrocil and Madacide 1 1/2 and then polyseptol 1.

Table (17): Minimal inhibitory concentration (MIC) of different types of disinfectants against gram negative bacilli.

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Concentration of Disinfectant</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration Level</td>
<td>1/2</td>
</tr>
<tr>
<td>Alkanol</td>
<td>Conc. Solution</td>
<td>-</td>
</tr>
<tr>
<td>Polyseptol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Clorox</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydrocil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mada-cide 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cidex</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Fig. (14): Minimal inhibitory concentration (MIC) of different types of disinfectants against Gram negative bacilli.
The MIC of different disinfectants versus candida isolates are illustrated in Table 18 (Fig. 15).

- The most potent disinfectants working against candida were [cidex and clorox 1/16] followed by [alkanol and hydrocil 1/8], Mada-cide 1 1/4 and lastly polyseptol 1/2.

Table 18: Minimal inhibitory Concentration (MIC) of different types of disinfectants against Candida.

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Concentration of Disinfectant</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. Solution</td>
<td>1/2</td>
</tr>
<tr>
<td>Alkanol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polyseptol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Clorox</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydrocil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mada-cide 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cidex</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 15: Minimal inhibitory Concentration (MIC) of different types of disinfectants against Candida.
The effect of different disinfectants against the growth of Mycobacterium tuberculosis is illustrated in Table (19).

Cidex, hydrocil, clorox and alkanol were the most effective on the inhibition of the growth of Mycobacterium tuberculosis, while polyseptol and Mada-cide 1 were not effective on the inhibition of growth of M. tuberculosis.

Table (19): The effect of different disinfectants in the inhibition of the growth of Mycobacterium tuberculosis.

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkanol</td>
<td>-</td>
</tr>
<tr>
<td>Polyseptol</td>
<td>+</td>
</tr>
<tr>
<td>Clorox</td>
<td>-</td>
</tr>
<tr>
<td>Hydrocil</td>
<td>-</td>
</tr>
<tr>
<td>Mada-cide 1</td>
<td>+</td>
</tr>
<tr>
<td>Cidex</td>
<td>-</td>
</tr>
</tbody>
</table>

*+ growth*  
*- no growth*
Results

The MICs values of different types of antiseptics towards different organisms isolated from workers hands are illustrated in Table (20), Fig. (16).

Hydrocil was the most effective against S. epidermidis (1/8) followed by alkaonl (1/4), polyseptol (1/2) and lastly Madacide-1 (1).

Alkanol and hydrocil (1/2) were the most effective antiseptics against S. aureus followed by polyseptol (1) while Mada-cide 1 was not inhibitory of the growth of S. aureus.

The most potent antispetic against S. saprophyticus was hydrocil 1/16 followed by alkanol 1/8, polyseptol 1/4 and Madacide-1(1/2).

The most potent antispetic against S. haemolyticus was alkanol and hydrocil 1/16 followed by polyseptol 1/8 and Mada-cide 1(1/4).

Table (20): Minimal inhibitory Concentration (MIC) of different antiseptics against staphylococci isolated from workers hands.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Alkanol</th>
<th>Polyseptol</th>
<th>Hydrocil</th>
<th>Mada-cide 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis</td>
<td>1/4</td>
<td>1/2</td>
<td>1/8</td>
<td>1</td>
</tr>
<tr>
<td>S. aureus</td>
<td>1/2</td>
<td>1</td>
<td>1/2</td>
<td>Not affected</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>1/8</td>
<td>1/4</td>
<td>1/16</td>
<td>1/2</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>1/16</td>
<td>1/8</td>
<td>1/16</td>
<td>1/4</td>
</tr>
</tbody>
</table>
Fig. (16): Minimal inhibitory concentration (MIC) of different antiseptics against staphylococci isolated from workers' hands.
Results of bactericidal activity of different types of disinfectants using the British/European standard test method is illustrated in table (21).

Table (21): Results of bactericidal activity of different types of disinfectants using the British/European standard test method.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Staphylococci</th>
<th>MRSA</th>
<th>Pseudomonas</th>
<th>Klebsiella</th>
<th>Candida</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact time</td>
<td>2</td>
<td>5</td>
<td>10</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

**Disinfectants:**

<table>
<thead>
<tr>
<th></th>
<th>+ve</th>
<th>-ve</th>
<th>+ve</th>
<th>-ve</th>
<th>+ve</th>
<th>-ve</th>
<th>+ve</th>
<th>-ve</th>
<th>+ve</th>
<th>-ve</th>
<th>+ve</th>
<th>-ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Polyeptol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cidex</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clorox</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mada-cide l</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

+ve = growth  -ve = no growth

Alkanol is suitable for surface disinfection but it needs a higher contact time > 2 minutes to get ride of Pseudomonas and Candida (surface).

Cidex was effective at contact time > 5 minutes versus all microorganisms tested (soaking).

Hydrocil was highly effective against gram positive bacteria at a contact time 2 minutes (air).

**Results of in-use test:**

The in use-dilution of the cidex disinfectant taken from containers for contaminated instruments was sufficient for 14 days in killing all the organisms added as indicated by no growth in the 2 plates after the incubation period.
Discussion

In this study, however, the results obtained from air sampling, by the two methods, (exposure plates and RCS air sampler) show the evidence of contamination of air. This contamination of air is mostly referred to the increased number and movement of circulating staff within the ICU unit and this finding are in agreement with those of Zaza and Jarvis, (1996).

The colony count by strips in most cases (80%) was higher than that counted by plates, because of centrifugal force of RCS air sampler, which concentrates the air into the strip.

In this study the most common microorganisms isolated from environmental swabs (laryngoscopes, ventilators, air condition grills and beds) were S.aureus 44%, Gram negative bacilli 36% and lastly CNS was 20%.

The warm conditions in the ventilators and associated equipment, together with the moisture accumulated leads to colonization by large population of gram-negative bacteria particularly Pseudomonas aeruginosa and Klebsiella spp, which may produce fatal pneumonia in immunocompromized patients using such equipment (Zack et al., 2002).

Prevention of nosocomial pneumonia in critically-ill patients who are mechanically ventilated need care of respiratory therapy devices, which prevent cross contamination (Voss and Widmer, 1997).
Effective washing of workers hands can prevent nosocomial infections, particularly in high-risk areas of the hospital (e.g. ICU). The hands of health care workers are the main vehicles of multidrug-resistant bacteria and nosocomial infections in hospital wards (Wenzel, 1998).

The study of microbial flora of the skin include resident and transient flora. The resident microorganisms, which are integral part of normal skin. They live, grow and multiply in the superficial layers. Examples are CNS and diptheroids. While transient microorganisms are not an integral part of normal skin, but gain access to them from animate or inanimate surfaces. Examples of them are S. aureus and E. coli (Kim et al., 2003).

This agreed with our sampling of workers hands where the commonest microorganisms isolated was S. epidermidis (40%) followed by S. aureus (24%), S. saprophyticus (20%) and S. haemolyticus (16%) but E.coli was not found during this study.

Systemic review evidence, appraised and used by EPIC (European Prevalence of Infection in Intensive Care) guideline developers identified several well designed studies showing that patients contact resulted in contamination of health care workers hands by pathogens. For example, 10% of all patient-nurse interactions on the ICU resulted in transmission of Klebsiella to the nurse’s hands. However study of Pratt et al., (2001) showing that staff dressing wounds with MRSA have an 80% chance of carrying the organisms on their hands.
Girou et al., (2002) found that the correct choice for disinfection of the hands are alcoholic preparations especially that are rubbed in. These finding came in agreement with the finding of Stefanska et al. (2002) who found that S.aureus was resistant to QACs. Russo et al., (2003) optimized a new antiseptic formulation specifically targeted at skin, which is 5.0% chloramine-T diluted in 50% isopropyl alcohol with the efficacy against clinically important bacteria, fungi and viruses.

These finding are in agreement with our study since we found that the most effective antiseptics of the hands were alkanol and hydrocil while polyseptol and Mada-cide 1 were less effective.

On the other hand, the frequency of bacteria isolated from workers nose were S. aureus (50%) and S. epidermidis (50%), this finding was in accordance with Volk et al, (1991), who stated that S. aureus was found in the nasopharynx of 20-30% of adult at any time, and this may increase to 50-70% carriers rate in a hospital setting.

As regard patients swabs, 66% of isolated organisms from patients nose were gram-positive cocci. S. aureus was 40% of isolated gram-positive cocci and 34% were gram-negative bacilli.

This finding was near to the finding of Volk et al., (1991) who found that S. aureus was found in the nosopharyngeal at 50-70% carrier rate in a hospital setting.
In this work, the most common isolate of rectal swabs from patients was E-coli.

Contribution to the seriousness of nosocomial infections, especially in ICUs, is the increasing incidence of infections caused by antibiotic-resistant pathogens, so surveillance of antibiotic resistance is important in ICU (Hanberger et al., 1999).

Nosocomial infections, especially those caused by antibiotic resistant pathogens, represent an important source of morbidity and mortality for the patient hospitalized in an ICU. Important antibiotic-resistant nosocomial pathogens include Methicillin Resistant S. aureus (MRSA), vancomycin-resistant enterococci (VRE), gram-negative bacilli (especially, Klebsiella and Enterobacter) producing extended-spectrum β-lactamases (ESBL), multiple drug-resistant Mycobacterium tuberculosis, and fluconazole-resistant Candida sp. The key to control of antibiotic-resistant pathogens in the ICU is vigorous adherence to infection control guidelines and prevention of antibiotic misuse. Antibiotic restriction policies clearly result in reduced drug costs. Evidence suggested that reducing use of certain antibiotics may lead to a decreased prevalence of antibiotic-resistant pathogens: vancomycin, VRE; gentamicin, gentamicin-resistant gram-negative bacilli; and ceftazidime, gram-negative bacilli producing extended-spectrum β-lactamases. Limited data suggest that measures to control antibiotic use do not adversely affect and may actually improve patient outcomes (eg, decreased length of stay, risk of subsequent infection) (Jarvis, 1996).
The development and use of antibiotics for the chemotherapy of bacterial infections was one of the most remarkable accomplishments in medicine of the 20th. However, antibiotic-resistant bacteria were found in clinical isolates soon after the introduction of the earliest antimicrobial agents into the market. Resistances to antimicrobial agents are serious among immunocompromised host. The most important of these organism are MRSA, VRE, ESBL. These resistance have made anti microbial therapy of many infections extremely difficult or virtually impossible in some instances (Yamagnchi and Ohno, 2001).

Surveillance of bacterial resistance is a key element in understanding the problem. The large number of existing networks for resistance surveillance need to be coordinated and the results made available. To help doctors choose appropriate antibiotics and to detect local epidemics of resistant bacteria surveillance at local level is necessary. Good quality local data provide a basic for national and international surveillance (Brossete et al., 1998).

MRSA spread rapidly within hospital and can cause substantial morbidity and mortality (Marchese et al, 2000). We must know well the main mode of transmission of MRSA to control it. Hands of heath care-workers which may be contaminated by contact with colonized or infected patient, colonized or infected body sites of personnel themselves, or devices or environmental surfaces contaminated with body fluids containing MRSA (Ayliffe, 1996).
In this study the percentage of MRSA from workers' hands was 8.3% of isolated S. aureus, and 40% of workers' nose.

However, a study done by Al-Hendy et al. (1997) in Benha University Hospital found that (62.5%) of S. aureus isolated from nasal samples was MRSA.

In patients group, 80% of S. aureus isolated from nasal samples was MRSA. However, our results were not in agreement with that of Lucet et al. (2003), they found that percentage for MRSA carriage in nasal swab from ICU patients was 20%, but they also mentioned that, there are many factors which increase this percentage, as age older than 60 years, prolonged hospital stay in transferred patients and surgery. This may explain our results, because most of patients present in ICU were referred from other wards.

In this study, 100% of MRSA were sensitive to vancomycin and this finding was in agreement with Duckworth, (1993). They found that all MRSA were vancomycin sensitive.

This finding was also in agreement with the results of Rasha (2000) who reported that vancomycin was the most effective antibiotic against gram-positive isolates particularly S. aureus.

In this work imipenem was the most effective antibiotic for gram-negative bacilli isolated from rectal swabs of patients.
These results were consistent with the data of *Rasha (2000)* who reported that imipenem proved to have a broad spectrum and a high activity against gram-negative bacilli.

Since, large number of gram-negative isolates from rectal swab in our study were suspected or confirmed ESBL producers, 62.9% of E-coli, 50% of *K.ornithinolyticus*, 40% of both "*K.oxytoca*, and *Proteus mirabilis*" and 20% of *Citrobacter koseri*. Imipenem was very effective in all ESBL-producing gram negative bacilli.

These results are also in agreement with that of *Bell et al. (2002)* they found that the best coverage against ESBL producing isolates was obtained with imipenem.

Sterilization and disinfection of patient care items are an important aspect of infection control. Every health care establishment should have a disinfection policy depending on the services provided, types of items used and facilities available (*Ayliffe et al., 1990*). Disinfection describes a process by which pathogenic organism with the exception of bacterial spores are killed, in order to prevent their transmission and is achieved by action on their structure or metabolism (*Russel et al., 1982*).

Disinfection policy is one step in infection control program in hospital, which is very important especially in ICU, because patients in ICU are more susceptible to nosocomial infections and they are exposed to great hazards of contamination and cross infection than others in ordinary wards. This is due to they receive much more
nursing attention and various forms of instrumentation (Spatenkova et al., 2002).

The disinfection policy in the ICU should provide information on all types of disinfectants and on their procedure for their use which depend on the degree of risk of infection involved in the use of the item, whether critical, semi-critical or noncritical (Rutala, 1987).

In this study various chemical disinfectants and antiseptics were tested for their activity against different microorganisms isolated from ICU. The chemical disinfectants and antiseptics which were tested included: alkanol, polyseptol, cidex, clorox, hydrocil, and mada cide l and their activity were evaluated by using microtitration and tube neutralization methods.

In our study, the effectiveness of the various disinfectants and antiseptics versus inhibition of growth of environmental isolates was assayed.

In air samples, hydrocil, Mada-cide 1, alkanol, and cidex were effective in the inhibition of the growth of microorganisms isolated from air, while polyseptol was not effective.

In this study, swabs were taken from laryngoscope, ventilators and air condition, the cidex, clorox, hydrocil, alkanol and Mada-cide 1 were effective in inhibition of growth of microorganisms, while polyseptol was not effective.
In bed swabs, all used disinfectants were effective in the inhibition of growth of microorganisms.

The number of antiseptics and disinfectants tests described in the world, show that, there is a lack of agreement among workers in different countries on standardization of the component of the testing method. The performance of different testing procedures yields a diversity of results for the same disinfectant. Disagreement is still on the testing method themselves, varying results will be obtained (Favero and Bond 1991).

In addition we did laboratory testing of different disinfectants and antiseptics versus Staphylococci, gram-negative bacilli, Candida and Mycobactrium tuberculosis by using microtitration and tube neutralization tests.

In Staphylococi, cidex was the most potent (1/16), followed by clorox (1/8), Mada-cide 1 (1/4), ployseptol (1/2) and lastly hydrocil and alkanol (1). While, the most potent disinfectant working against gram-negative bacilli were cidex and clorox (1/8), followed by alkanol (1/4), Mada-cide-1 (1/2), hydrocil (1/2) and then polyseptol (1). The most potent disinfectants working against candida were cidex and clorox (1/16) followed by alkanol and hydrocil (1/8), Mada-cide 1 (1/4) and lastly polyseptol (1/2).

The results are in agreement with that of McDonnell and Russell (1998), they found that most effective disinfectants were cidex and clorox against gram-positive cocci, gram-negative bacilli and Candida.
In our study the effect of different disinfectants against the growth of Mycobacterium tuberculosis, was studied, cidex, hydrocil, clorox and alkanol were the most effective in the inhibition of growth of M. tuberculosis, while polyseptol and mada cide-I were not effective in the inhibition of growth of M. tuberculosis.

The resistance of Mycobacterium tuberculosis to disinfectants has been considered intermediate between those of other vegetative bacteria and spores. This is attributed in part to their unusually higher cell wall lipid content and resultant hydrophobicity (Russel et al., 1986).

The results of disinfectants effect in Mycobacterium tuberculosis in our study are also in agreement with that of Best et al. (1990). They found that QACs and iodophor were completely ineffective, chlorohexidine and cidex have good effective.

Patients with critical illness requiring aggressive medical intervention because they are at risk of acquiring serious nosocomial infection that may lead to increases in medical expenditures, morbidity, and mortality. Infection control of this population entails continuos surveillance for HAI with investigation of outbreaks. Policies for effective antibiotic utilization, disinfection of medical devices and hospital environment, and patient isolation may limit nosocomial infection in this population (Dieckhaus and Cooper, 1998).

In this study, data collection concerning types of microorganisms isolated from personnel, patients, & different sites in the ICU, and their antimicrobial & anti-septic resistance patterns were only a preliminary
step in building-up an integrated strategy to control infection in Benha university hospital ICU, and to give model to other hospitals & infection control teams to get benefit from it. Another aim was reached in this work, is that a bridge between laboratory and ICU workers was built up for the purpose of patient’s benefit. Simple guidelines about changing behaviour of the ICU personnel and their awareness about the potential danger imposed on their patients, and on themselves were among our goals, & further studies are needed to follow-up and assess the clinical outcome of these behavioural and conceptual changes.

Also strict measures about anti-septic and disinfectants use were suggested including appropriate choices and methods and timing of their use, antimicrobial regimens were revised meticulously with clinicians. Rotational policy of use of main antibiotic families was advised. 1st line & 2nd line concept was established, to save newer and effective drug, for those cases with multiple drug resistance.
SUMMARY
SUMMARY

Infection is one of the principal hazards to which patients in critical care centers are exposed. In addition to being more susceptible, they are exposed to great hazards of contamination and cross infection than most patients in ordinary wards. This is due to they receive much more nursing attention, handling and various forms of instrumentations in particular tracheostomy, mechanical ventilation, aspiration of bronchial secretion, catheterization of urinary tract, treatment of open wounds and prolonged intravenous infusion.

The commonest microorganisms causing disease in critical care units are gram negative bacilli and staphylococci. Gram negative bacilli encountered in critical care units include: Enterobacteriaceae (E. coli, klebsiella spp., proteus spp., enterobacter spp., and serrata spp.), pseudomonas aeruginosa, hemophilus influenza and bacteroids fragilis. These organisms are known as opportunistic pathogens, they are normally harmless commensals, but they are able to multiply in tissues and cause diseases in immunocompromized patients.

The aim of this study is to put a strategy for infection control in the ICU in Benha University Hospital.

This study comprised collection of microbiological samples from environment, patients and working staff in intensive care unit in Benha University hospital.
The environmental samples include:
- 20 samples from air.
- 20 samples from ventilator.
- 20 samples from air condition grill.
- 20 samples from laryngoscope.
- 20 samples from beds.

Workers samples:
- 50 hand samples.
- 50 nasal samples.

The patients samples include:
- 50 nasal samples.
- 50 rectal samples.

All specimens were subjected to the following:
1. Isolation of the organisms by culturing on ordinary bacteriological media (blood, chocolate and MacConkey agar) and incubating aerobically at 37°C for 48 hours.
2. Gram stained smear for the isolated pathogen.
3. Biochemical reaction of the isolates.
4. Antimicrobial susceptibility test for the isolated pathogen.
5. The identified isolates were tested for the action of disinfectants by microtitration method. and tube microbicidal method.
Identification of gram negative bacilli was done as follow:

Biochemical identification:

a) Oxidase test.

b) API 20 E.

Anti microbial susceptibility testing.

By conventional disc diffusion methods on Muller Hinton agar for detection of (ESBL).

Identification of Gram positive cocci was done as follow:

- Biochemical identification
  a- Catalase test: to differentiate staplylococci (+ve) from streptococci (-ve).
  b- Coagulase test: to identify staph aureus.
  c- Dnase test: to identify staph aureus.
  d- API staph.

- Antimicrobial susceptibility testing:

  Conventional disc diffusion method on Muller Hinton agar for detection of MRSA.

In this study, the results obtained from the air sampling, by the two methods, show the evidence of contamination of air.

In this study, samples were taken from laryngoscope, ventilators and air condition samples; the cidex, clorox, hydrocil, alkanol and Mada-
RECOMMENDATION
RECOMMENDATION

In the light of the results of this thesis, it is recommended to:

1- For disinfection of air, hydrocil is recommended as the first choice in ICU, followed by Madacide 1, and alkanol.

2- For disinfection of laryngoscopes, hydrocil is recommended as the first choice in ICU, followed by alkanol and clorox.

3- For disinfection of ventilators, cidex and clorox are recommended followed by hydrocil and alkanol.

4- For disinfection of air condition grill, hydrocil and clorox are recommended followed by cidex and madacide 1.

5- For disinfection of beds, hydrocil, cidex and alkanol are recommended followed by madacide 1 and clorox.

6- Use of hydrocil and alkanol as the antiseptic is the first choice in hand as antiseptic.

7- Combating the incidence of nosocomial drug resistance, such as MRSA, ESBL. By restriction of newer drugs for multiple resistant organisms, and to follow-rotational policy by use of one family of drugs for a period, then shifting to another family.

8- Making regular timed surveillance to assess the microbial ecology, and emerged resistance in every hospital.

9- Making connection between clinicians and ICU workers from one side, and infection control team from the other side.
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ARABIC SUMMARY
المؤشر العربي

تمثل العدوى المكتسبة من المستشفى خطراً كبيراً في الهيئات الصحية المختلفة، وآثارها ملموسة على المرضى وعائلاتهم وأجهزة الرعاية الصحية.

المريض في وحدات العناية المركزة يكون أكثر تعرضاً لهذه العدوى المكتسبة وذلك لأنهم يتعرضون لاستخدام كثير من الأجهزة بالإضافة إلى ضعف مناعة هؤلاء المرضى.

هناك ازدياد مضطرد في مقاومة الميكروبات المسببة للعدوى في المستشفيات لمختلف المضادات الحيوية والتي تعتبر الخط الأول لعلاجها وهذا يتطلب استخدام أنواع أحدث للمضادات الحيوية بتكالفة أعلى مما يسبب عيباً مادياً على أجهزة الرعاية الصحية وعلى المرضى وأسرهم.

وتهدف هذه الرسالة إلى وضع خطة لمكافحة التلوث في قسم العناية المركزة المستشفى بنها الجامعي وذلك بأخذ عينات من هواء البيئة المحيطة بالمرضى وكذلك من الأسرة والأجهزة التي تم استعمالها بواسطة المرضى.

كما تم أيضاً أخذ عينات من المرضى بواسطة مسحات من الأنف والشرج وعينات من العاملين بواسطة مسحات من الأنف واليد وذلك للكشف عن حامل الميكروبات المقاومة للمضادات الحيوية.


وджعت هذه العينات للاختبارات التالية:
- عزل الميكروب على الأوساط البكتريولوجية المعتادة.
- فحص أفلام مصبوغة للتعرف على الميكروب.
- اختبار الحساسية باستخدام أفراد المضادات الحيوية.
الخصوص (العربي)

تمثل العدوى المكتسبة من المستشفيات خطراً كبيراً في الهيئات الصحية المختلفة، وآثارها ملموسه على المرضى وعائلاتهم وأجهزة الرعاية الصحية.

المريض في وحدات العناية المركزية يكون أكثر تعرضاً لهذه العدوى المكتسبة وذلك لأنهم يتعرضون لاستخدام كثير من الأجهزة بالإضافة إلى ضعف مناعة هؤلاء المرضى.

هناك ازداد مضطرد في مقاومة الميكروبات المسببة للعدوى في المستشفيات لمختلف المضادات الحيوية والتي تعتبر الخط الأول لعلاجها وهذا يتطلب استخدام أنواع أحدث للمضادات الحيوية بتكلفة أعلى مما يسبب عيب مادي على أجهزة الرعاية الصحية وعلى المرضى وأسرهم.

وتهدف هذه الرسالة إلى وضع خطة لمكافحة التلوث في قسم العناية المركزية المستشفى بنها الجامعي وذلك بأخذ عينات من عينات البصيلة المحيطة بالمريض وكذلك من الأشرار والأجهزة التي تم استخدامها بواسطة المريض.

كما تم أيضاً أخذ عينات من المريض بواسطة مسحات من الأنف والشرج وعينات من العاملين بواسطة مسحات من الأنف واليد وذلك للكشف عن حامل الميكروبات المقاومة للمضادات الحيوية.


وخضعت هذه العينات للاختبارات التالية:

- عزل الميكروبي على الأوساط البكتيرولوجية المعتادة.
- فحص أفلام مصبوغة للتعرف على الميكروب.
- اختبار الحساسية باستخدام أنواع المضادات الحيوية.
بالإضافة إلى عملي الآتى:

• بالنسبة للعينات التي أخذت من هواء البيئة المحيطة بـ المرضى والأسرة والتكيفات والأجهزة المستخدمة بعضاًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًًٍ
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بالنسبة للبكتيريا المسببة للدرين:

الدرين كان أكثر تأثيراً على تقل ارتفاع البكتيريا ونسبة الفنين.

من خلال هذه الدراسة أيضاً:

كان ميكروب استفانت (S. epidermidis) أعلى الميكروبات التي تم العزل منها من أدنى العاملين، بليا ميكروب استفانت أوريس (S. aureus)،

وكانت نسبة استفانت أوريس مقاومة للميثيسيلين في هذه المجموعة (32%).

وكان ميكروب استفانت أوريس مساوايا ليكروب استفانت أوريس (50%) من أدنى العاملين.

وكانت نسبة استفانت أوريس مقاومة للميثيسيلين في هذه المجموعة (40%).

وكانت نسبة عزل الميكروبات الكروية العنقودية إيجابة الجرام من عينات أدنى المرضى (34%)، بينما الميكروبات العصوية سالبة الجرام كانت (60%).

ومن هذه المجموعة كانت نسبة استفانت أوريس مقاومة للميثيسيلين (80%).

وقد وجد أن الانتكاسين هو أكثر المضادات الحيوية فاعلياً تجاه استفانت أوريس بنسبة (100%).

بينما كانت الإيكولاي (E. coli) أكثر الميكروبات العصوية سالبة الجرام عزلاً من عينات الشرج من المرضى،

وكانت نسبة كبيرة منها مقاومة للمضادات الحيوية

كان أكثر المضادات الحيوية فاعلياً ضد مجموعة الميكروبات المعوية

هو الأمبيرين (Enterobacteriaceae)
ومن خلال هذا البحث يتبين أن:

- أفضل مطهر يمكن استعماله في تطهير الهواء هو الهايبروسيل ويتبعه الماداسيد 1.
- أفضل مطهر يمكن استخدامه في تطهير منظار الحنجرة هو الهايبروسيل والالكانون.
- أفضل مطهر يمكن استخدامه في تطهير جهاز التنفس الصناعي هو السايدكس والهايبروسيل.
- أفضل مطهر يمكن استخدامه في تطهير أجهزة التكييف هو الهايبروسيل الكلوركس.
- أفضل مطهر يمكن استخدامه في تطهير الأسرة هو الهايبروسيل والسايدكس والالكانون.
- الالكانون والهايبروسيل أفضل المطهرات استفادةً لتطهير البيئة.

يجب أن يكون هناك تعاون بين الأطباء المعالجين وفريق مكافحة التلوث لعمل خطة لمنع ظهور الميكروبات المقاومة للمضادات الحيوية.
دراسة ميكروبيولوجية لوضع نظام مكافحة التلوث في قسم الحماية المركزية

بحث مقدم بحوثي للحصول على درجة الدكتوراه
في الباثولوجيا الإكلينيكية والكيميائية

محمود طلبة
الطبية/ صفاء إبراهيم عباس منصور

الاستاذ الدكتور / فتنت محمد طلبة
أستاذ الباثولوجيا الإكلينيكية والكيميائية
كلية طب بنها-جامعة الزقاقيق

الأستاذ الدكتور / سهير عبد الرحمن عبد السميع
أستاذ الباثولوجيا الإكلينيكية و الكيميائية
كلية طب بنها-جامعة الزقاقيق

الدكتورة / نجوى عبد الغني خميس
مستشار مساعد الباثولوجيا الإكلينيكية بالمستشفى التخصصي-جامعة عين شمس
كلية الطب بنها
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