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A Comparative Study of Domestic and Hospital Environmental Microbial Populations in Al-Ain (UAE)

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بسم الله الرحمن الرحيم

A COMPARATIVE STUDY OF DOMESTIC AND HOSPITAL ENVIRONMENTAL MICROBIAL POPULATIONS IN AL-AIN (UAE)

By

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ABSTRACT

This is the first attempt to estimate biological indoor pollution in the environment of Al-Ain city. The numbers and types of bacteria and fungi in the air and on the surfaces were measured in Al-Ain hospital and three different types of domestic environments.

Five different types of wards at Al-Ain hospital, medical, surgical, pediatrics, operating theater, and intensive care unit were studied. Their estimated indoor bioaerosols were compared to indoor bioaerosols in three types of dwelling houses, very good, average, and poor quality houses in Al-Ain city. A bacteriological mechanical air sampler, MK2 (Casella London) was used in this study.

The result of this study showed that the same groups of bacteria and fungi isolated from the hospital environment were also found in domestic air samples. The highest number of bacteria in the hospital was found in the pediatric and female medical wards while the lowest were in the operating theater. The number of bacteria in the domestic environment was related to the type of housing; the higher the quality of house the lower the number of micro-organisms.

Pathogenic and human related micro-organisms were found to be more prevalent in a hospital environment than in the domestic environment. In general the hospital air microbial counts were comparable to very good quality houses. The commonest species of fungi found in both environments were *Aspergillus niger.* Surface samples in hospital and homes showed that surface micro-organisms originated from air contaminants.

A comparison of hospital and domestic bacterial sensitivity was carried out using coagulase negative *Staphylococci* (CNS). These were also compared to the patients'n CNS. The sensitivity pattern of CNS indicated that the "environment" or the source of the microbes had some effect upon the micro-organisms. Domestic airborne CNS were very sensitive to nearly all the antibiotics tested while patients harbored the most resistant CNS with hospital airborne CNS falling in between. Hospital airborne agents would seem to be a mixture of patients' strains and the environmental strains possibly brought in by visitors to the hospital.

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

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CHAPTER I

INTRODUCTION

Pollution is one of the most pressing problems of our age. Pollution of the atmosphere has now reached a level that poses a potential threat not only to the health and well-being of populations of the globe but to the survival of entire life (Samet and Spengler, 1991).

This study focuses on indoor air, because indoor air is the medium through which people, buildings, and climate interact. Human health and their well-being are determined by physical, chemical, and biological properties of their indoor air; and indoor air quality can be readily defined and rationally controlled.

The contamination of the indoor environment by microbial population and other pollutants is a major health problem (Langmuir, 1980). The concentration of indoor pollutants varies with the strength of the pollution sources, the volume of the polluted space, the rate of air exchange between indoor and outdoor air, and other factors that affect removal of pollutants. Health risks from indoor pollution

depend on indoor pollutants concentration, patterns of human activity and type of indoor source of air pollution used such as combustion source, which, along with indoor concentration of pollutants, determine personal exposures (Samet and Spengler, 1991)

Indoor biological pollution, while of serious concern to all who inhabit interiors, has only recently began to receive the attention afforded outdoor or even indoor chemical pollution (Yunginer *et al.*, 1976). This apparent lack of interest is tied to the difficulties of sampling biological aerosols and their variable health effects. The airborne bioflora is inherently complex and variable to a point that defies quantification. The air in a single room in a "clean" house may contain hundreds of different kinds of biological particles and technology does not exist to quantify or qualify all of them.

As many as four distinct sampling modalities are required if those particles that are measurable are to be accurately assessed. Health effects of biological aerosols are basically different from those of their chemical counterparts. The majority of

bioaerosols are nonpathogenic and cause disease only in sensitized or grossly immunocompromised people. Furthermore, pathogenic environmental micro-organisms are usually able to infect only very susceptible hosts. The levels of either saprophytes or pathogens required to cause disease differ with each particle type and these levels are unknown for most microbes.

It is well documented that the hospital environment is a source of hospital acquired infections (Bennett and Brachman 1992). Many of the microbes which cause infectious diseases such as tuberculosis, staphylococcal infections, brucellosis and most of nosocomial pneumonias are transmitted by indoor air.

For this reason knowledge of the incidence of micro flora in the hospital and in residential dwelling is very important for the understanding of the possible types of infections and allergies caused by them. Furthermore, controlling the microbes in the hospital and domestic environment could play a role in the prevention of infectious diseases.

The aim of this study was to compare the numbers and types of airborne microbial population in a hospital and in three types of domestic environments in Al-Ain city. In doing this we were trying to find the relation between these two environments and the effect of the environment on the types, numbers of micro-organisms.

THE OBJECTIVE OF THE STUDY

The main purpose of the study was to compare the numbers and types of environmental micro-organisms in Al Ain hospital and those found in homes of different social strata in Al-Ain city.

It is well known that a hospital harbors a diverse population of micro-organisms. The micro-organisms in the hospital environment could be a source of hospital acquired infection. It has been documented that micro-organisms which cause infectious diseases can be airborne or be transmitted by handlers. Many studies have been carried out to estimate hospital environmental micro-organisms in different areas of the world. However, to our knowledge no microbial studies have been done in any of the Al-Ain hospitals or anywhere in the UAE.

The micro-organisms in the air environment or surfaces of homes at Al-Ain city have also not been documented. The influence of domestic microbes to inhabitants can be related to

infection in their own home. Some of these agents are transmitted by air, particularly from infected persons occupying the same room. Although most of these are viral upper and lower respiratory tract infections, bacteria can also be similarly transmitted, leading to for example streptococcal pharyngitis and diphtheria. It is essential to know the types of micro-organisms in the home environment and the extent of pathogenic micro-organisms frequently encountered in the air or on surfaces in the home environment.

If similar pathogenic organisms are found in the hospital and homes then they would be identified and their characteristics compared to estimate possible relationship and their movements between the two environments. The micro-organisms of importance to human health would be subjected to antibiotic sensitivity tests to determine their resistance patterns variation in both the hospital and home environments. The effect of the environment on any queried antibiotics resistance will be discussed.

CHAPTER II

1. REVIEW OF LITERATURE

1.1 History of Indoor Pollution

The problems of indoor pollution began with the energy conservation just before World War II, but the energy crisis of 1973 exacerbated the problems. In order to minimize the use of energy, several countries began looking for ways to conserve energy. The largest user of energy was found to be the building sector which adopted two ways of energy saving. First, the equipment used in buildings were made more efficient; and secondly, the buildings were better insulated to stop any air leaks, and used as little outside air as possible. The result of this process was less outside air entering into these buildings to sweep out pollutants that are generated within the building. Furthermore, occasionally pollutants brought in from the out door add to those already generated inside. Thus indoor levels of air pollution is usually considered worse than outdoor levels (Miller and Keller, 1991). Although this practice saved great amounts of energy, it was at an environmental cost.

Lucretius, a great philosopher, first suspected indoor air to be a potential agent for transmitting infectious disease, nearly two thousand years ago. He saw dust motes in a sun beam in a dark room and considered the possibility that the motes might carry pestilences (Gregory, 1961). Since then, it has been well documented that some diseases are transmitted by airborne particles (National Research Council 1961, 1981, Kundsin, 1980). Public interest of the indoor air pollution has grown considerably over the past few years. A large part of this interest began when Legionnaire's Disease was discovered in 1977 (Miller and Keller 1991). The indoor environment can potentially place human occupants at great risk, because enclosed spaces can confine aerosols and allow them to build up to infectious levels (Spendlove and Fannin, 1983). Ventilation systems can pick up contaminated air and distribute infectious micro-organisms to other parts of the building (Huddleson and Munger, 1940). Components of ventilation systems can actually become contaminated and pathogenic micro-organisms such as *Legionella pneumophila* are subsequently transmitted to the buildings occupants (Glick, 1978).

1.2 The Pollutants

The airborne pollutants that may be present in the indoor environment comprise a complicated mixture of living and inanimate materials of gases, vapors, fibers, dusts, and microbes. Studies carried out by Healthy Buildings International (Binnie, 1991) found that in over one third of more than 400 buildings studied, the major pollutant was allergenic fungi. In greater than two thirds, air supply systems were contaminated with dust, dirt and microbes. The microbiological contaminants of indoor air include viruses, bacteria, protozoa, algae, insect fragments, animal dander, and fungal spores. The contaminants may be viable organisms that multiply in an infected host or they may live in dust, soil, water, oil, organic films, food, vegetable debris, or wherever the micro climate provides the right temperature, humidity and nutrients for growth (Samet and Spengler, 1991). This investigation excludes viruses, algae, and parasites. Although these are important, they are outside the scope of our study

1.2.1 Dust

Clean ambient air at the breathing level (1.5 metres above the ground) contains 20-40 mg/m^3 of dust in the form of sea spray, soluble salts, organic matter, and microbes. Much of inhaled dust is harmless (Holt, 1980).

Indoor dust contains the same ingredients as ambient dust, but the composition is significantly different. House dust, especially in bedrooms, frequently contains mites, hairs and feces of pets, insect fragments, skin scales of human and pets. House dust may also contain pollen carried by wind or by insects (Meyer, 1983).

House dust may also contain fungal spores, and bacteria which can act as indirect allergens by stimulating the release of mediators from the host cell (Meyer, 1983), and other components such ash of cigarettes or incinerators, fibers, fingernail filings, food crumbs, glue, oil, soot, paint chips, plant parts, soil, wood shavings and others (Hung, 1994).

1.3 Sources of Indoor Airborne Microbes

1.3.1 Outdoor Environment:-

The majority of biological particles found indoor comes from the outdoor environment. The majority of fungal spores encountered indoors are derived either directly (by penetration) or indirectly (by penetration and subsequent growth on surfaces) from outdoor air. Most fungi are primary decay organisms and are abundant on dead or dying plant and animal materials. Many are plant pathogens and can be present abundantly in air wherever their host plants are grown. A few fungi are human pathogens. However, many fungal species are primarily saprophytic decay organisms that can opportunistically cause human disease and mainly exist in outdoor environments such as *Cladosporium*, *Alternaria, Pencillium* and *Aspergillus*. Usually outdoor fungal spore levels exceed those indoors, only in cases of serious contamination do indoor spore levels exceed outdoor levels. A wide variety of bacteria, most of which are not human pathogens such as *Bacillus* species and *Actinomycetes*, are abundant outdoor. These agents when provided with substrates after penetration may possibly grow on interior surfaces. Other bacteria

such as *Legionella pneumophila* which cause human disease thrive in outdoor reservoirs. This organismt is basically soil borne, and is probably introduced into cooling towers and other enrichmed environment during excavations for roads or buildings or through sand storms (Burge and Fealey, 1991).

1.3.2 Indoor Contamination:-

Micro-organisms found in the environment are associated with human droplets, dust particles or on skin squames. Droplet nuclei are the smallest particles of bacterial or viral residues from the evaporation of larger particles expelled by coughing, sneezing or talking. They may remain suspended in air for long periods and are carried by air current until inhaled or vented (Kundsin, 1985). Dust particles that have settled on surfaces may become resuspended by physical action and may remain airborne for a prolonged period. Airborne particles may remain suspended for hours or possibly days, depending on environmental factors. Skin squames may become airborne and provide a mechanism for the airborne transmission of organisms such as *Staphylococci* (Bennett and Brachman,. 1992).

Indoor buildup of bioaerasols results from two general processes:- material being shed by residents and accumulating indoors, and actual growth on interior substrates. Shed materials include, particulates such as human and animal skin scales (dander), bacteria, and dermatophytes. Human (or animal) skin scales, for example, support not only a lively mite population, but mesophilic bacteria and fungi such as *Aspergillus amstelodami* and *Wallemia sebi,* both of which can stand relative dryness. Skin scales as well as many fabrics, leather and wood materials, absorb sufficient water from the air to support fungal growth.

Bacterial endotoxins and (theoretically) fungal mycotoxins can accumulate indoors from micro-organisms growing on interior surfaces. Adverse health effects from endotoxin in commercial environments have been reported (Aas, 1980). Mycotoxins have not been measured in air, but have been implicated in cases of leukemia following exposure to *Asperigillus* species. Most severe indoor biological pollution problems result from microbial growth on surface within structures. Any substrate that includes carbon source and water will support the growth of some micro-organism.

An absolute requirement for all kinds of interior contamination is a constant source of moisture, moreover a very small leak in a roof or water pipe is sufficient to support abundant fungal growth.

Modern appliances such as humidifiers, vaporizers, water sprays, conditioners, evaporative coolers, self-defrosting refrigerators and flush toilets, provide standing water reservoirs which, when not absolutely clean, can provide ideal enrichment situation for the growth of micro-organisms. In these appliances water (usually contaminated) is in contact with a moving air stream which can pick up small particulates (bacteria and antigens) and spray them in to room air (Burge, 1985).

1.4 Factors Influencing Indoor Microbial Levels:-

Indoor surface contamination by bacteria, fungi or other biological particles is only dangerous when the particles become airborne and inhaled. There are several factors that can cause aerosolization of surface micro-organisms as listed below:

1.4.1 Air Currents

Air is almost never still, and even the most delicate of air currents can cause fungal spores such as those of *Aspergillus* and *Penicillium* to become airborne. Air current produced by convection from radiant heat, and by air mechanically circulated by forced air systems are more than adequate to spread dust including entrapped biological particles, as well as mobilizing surface growth.

Practically any human or pet activity can increase airborne microbial loads. Such activities include, vacuming, sweeping dusting, scrubbing contaminated surfaces and bed making. Indoor concentrations vary, not only with the strength and the concentration of the pollution sources, but with the volume of the polluted space; and the rate of air exchange between indoor and outdoor air.

1.4.2 Other Factors

The size and density of the airborne particles can influence the distance it moves. Williams *et al.* (1956) discusses the multitude of factors influencing the bacterial count in the air of occupied school rooms. They list the following as important factors:

- Room air temperature versus the temperature outside the class room
- Relative humidity outside versus the relative humidity inside.
- Rainfall on the day of the visit and over the previous 7 days.
- Solar radiation on the day of the visit and on the previous 7 days.
- External wind velocity and the degree of window opening.
- Ventilation rate and the number of children in the room
- Amount of talking and the amount of activity of the children.

Wind direction and relative humidity were the best predictors of micro biological air concentration. The concentrations of bacteria and fungi were negatively associated with temperature (except for Actinomycetes and the indoor concentration of moniliaceous molds) and wind speed (except for *Basidiomycetes*) but were positively associated with relative humidity (Macher and Huang, 1991).

1.5 Type of Environmental Bacteria

Bacteria occur in almost every environment particularly in dusty, dirty places inhabited by humans or animals. Most of the species of bacteria isolated from buildings are harmless and frequently include members of the genera, *Bacillus* species, *Micrococcus* species and *Corynebacterium* species. However, the species that have been associated with diseases include *Pseudomonas* species especially *P.aeruginosa, Flavobacterium* species, *Staphylococcus aureus, Serratia marcescens* and *Legionella penumophila* (Binnie, 1991).

1.6 Type of Environmental Fungi

Fungi are ubiquitous in nature and inevitably enter buildings from the outdoors to set up colonies wherever conditions for growth are favorable. They are very resistant, and once they have established themselves in a niche in a building, they are very difficult to completely eradicate. Studies carried out by Healthy Building International (Binnie, 1991) found that in more than one third of the buildings studied, one of the major pollutants was fungi. Some of these fungi are known to be pathogenic. Fungi isolated from heating, ventilating, air conditioning systems and other parts of buildings can cause problems in susceptible people either by causing direct infection or by causing an allergy reaction (Miller and Keller, 1991)

1.6.1 Niche of Fungi in Indoor Environment

Some fungi, particularly some members of the Hyphomycetes, grow very well in an indoor environment. Species of *Penicillium*, *Aspergillus, Cladosporium, Phoma, Rhodotorula, Ulocladium, Stachybotrys,* etc. have been isolated from materials used in indoor environments. *Chaetomium* of the Ascomycotina

and some Basidiomycetes have also been isolated from wood structures of buildings. These fungi cause decays in wood. Many other fungi have also been isolated from or observed on plants and potting soils and on paper, plaster, carpet, glass, plastics, and many other items. Many fungal spores can also come from vegetation near buildings. These spores may be carried into a building through doors, windows or outside air intakes (Hung, 1994).

In over 200 surveys of airborne spores, carried out in various parts of the world, four genera; *Cladosporium*, *Alternaria*, *Pencillium* and *Aspergillus* accounted for the highest mean percentage. These four genera also constituted the most prevalent in allergic respiratory disease (Col and Samson, 1984). *Aspergillus* species are known to cause infections, especially *A. niger* and *A. fumigatus* (Binnie, 1991).

1.7 Hospital Environment

The purpose of estimating the types and numbers of bacteria in hospital air is as relevant as for other indoor microbial population. The microbial quantities and types are directly and indirectly related to patients as a source or as recipients. The role the hospital environment plays in nosocomial infections can occasionally be scientifically determined. Although hospital hygiene is generally high, it is impossible to exclude microbes from the environment; but the quantity can be controlled. Each hospital has different standard of cleanliness; this may influence patient care. There are no established standards for viable or non viable particulate counts in the operating room or in any other hospital areas. However the accumulation of micro-organisms on surfaces can be used to evaluate housekeeping procedures as well as micro-organisms shed by patients and personnel. The micro-organisms of the hospital environment and the microbiology of hospital acquired infections are inseparable. Health personnel have been reluctant to accept this fact, but finding a solution depends on acceptance of the problem (Kundsin, 1985).

Some knowledge of environmental microbes could serve as a tool for:-

- Identifying institutionally related infectious disease .
- Tracing movements of airborne bacteria .
- Detecting persons, objects, activities, or items of equipment that generate airborne contamination.
- Evaluating the efficiency of air cleaning devices or systems.
- Assessing the hazards of hospital ventilation systems .

Sayer *et al* (1972) in a study of hospital airborne bacteria, categorized the hospital bacteria into two groups :-

(a) The usually inconsequent airborne bacterial flora group which included the colonies of Gram positive rods, *Micrococcaceae*, *Diphtheroids*, *S.epidermidis* and alpha or gamma haemolytic *Streptococci.*

(b) The potentially pathogenic airborne bacterial flora group, which Included: *Streptomyces*, *Flavobacterium*, *Mimae tibe* e.g. *Mima* like or *Herellea* like, *Klebsiella*, *Staphylococcus*

aureus, *Achromobacter* group, *Pseudomonas species*, *Psudomonas aeruginosa* and possiblly *Erwinia*.

1.8 Source of Indoor Contamination in Hospital Environment

Indoor hospital microbes can accumulate like any other occupied buildings as described already for domestic buildings. Addition to these there are special considerations for the hospital sources:

1 Staff members can be a source of environmental micro-organisms because of the inhalation of ambient microorganisms, therefore, personnel who work in the cleanest hospital area such as operating room, have the lowest rate of colonization with *Staphylococcus aureus* and personnel who work in open wards have the highest rate of colonization (Kundsin 1985)

2 Patients and health care personnel can be victims as well as sources of environmental microorganisms. They are

victims when infected or colonized by hospital flora and sources when they shed these microorganisms.

3 Medical equipment and fluids when its contaminated.

4 Appliances such as humidifiers, cool-mist vaporizers, air conditioners and ventilation contribute to aerosols.

5 Visitors and whatever materials they bring with them such as food and flowers .

6 Although there is (as yet) no evidence that the presence of carpets in hospitals create an infection hazard , a large amount of dust is deposited in carpets, and these dust particles are combined with large number of airborne potentially pathogenic microorganisms (Maurer, 1985).

1.9 Hospital Bacteria Associated with Infections

Transmission of tuberculosis and staphylococcal infections in hospital environment are examples of airborne spread by means of droplet nuclei. *Staphylococci* are shed in great numbers attached to tiny skin scales. *Staphylococcus aureus* may cause infections of the respiratory system when inhaled, food poisoning when swallowed and wound sepsis when allowed to

enter an open wound (Maurer, 1985). Although most staphylococcal wound sepsis is a result of hand transmission, in one report, several post operative wound infections were said to have resulted from the airborne spread of *Staphylococci* from a staff member who remained at the periphery of the operating room throughout the surgical procedure. The only route for transmission of the organisms was through the air (Bennett *et al.*, 1992).

Esherichia coli is one of the common causes of infections of the urinary bladder. Some types of *E.coli (Enteropathogenic E.coli EPEC)* may be responsible for outbreaks of gastroenteritis among neonatal unit. Other bacteria responsible for hospital infections are *Klibsiella, Proteus* and *Pseudomonas*, and spore forming bacteria such as *Clostridium tetani* which is commonly found in the soil and enters the hospital in dust (Maurer, 1985).

Legionella pneumophila, an agent of serious pulmonary disease, enters the indoor environment from building vents located close to cooling towers, and also from building hot water

services (Morely, 1985). Formation of aerosols from contaminated water is a major mode of spread of Legionella (Hart and Makin, 1991), humans probably acquire the organism by inhaling aerosols generated from these environmental sources as there is evidence to suggest that inhalation is also a mode of entry.

1.10 Domestic Environmental Microbes:-

In previous studies (Kodama and McGee 1986; Raza *et al*., 1989; Macher and Huang, 1991) domestic environmental micro-organisms were generally categorized into bacterial and fungal groups .

A] Bacteria were conveniently divided into 5 groups as follows:

i) Gram positive cocci.

ii) Gram positive bacilli.

iii) Gram negative cocci.

iv) Gram negative bacilli.

v) Gram negative diplococci.

B] Fungal species of domestic indoor air have been identified as:- *Aspergillus, Alternaria, Cladosporium, Penicillium, Curvalaria, Mucor* and *Helminthosporium.*

The benefits of estimating the types and numbers of microorganisms in Domestic environment includes;

[a] Establishing the types of microorganisms in homes environment .

[b] Identifying the pathogenic and allergenic organism which occupies the home environment.

[c] Evaluating the sanitation of homes.

[d] Estimation the environmental factors affecting the presence of microorganisms in homes

[e] Determining the relationship between the home social strata and the micro-organisms present.

1.11 Sick Building Syndrome (SBS)

In 1982 the World Health Organization recognized the "sick building syndrome" as a malady affecting a proportion of people in certain problem buildings. The indicators that a building may be sick usually are increased staff complaints of minor health symptoms, stuffy air, intermittent odors, and visible increases in dust levels. Other factors may be uneven temperature zones, noticeable smoke accumulation, and dirt coming out of air supply diffusers. Management may be aware of increased staff absenteeism and reduced productivity. The symptoms that affected people are usually groups of almost trivial problems such as eye, nose and throat, irritation; headache, rhinitis and sinusitis with skin irritation; cough, shortness of breath, and general lassitude; and dizziness, nausea and mental confusion. The study of sick building syndrome is on going, and as methods continue to be developed, more will be learned about the true role of microbes and their products in the problem (Binnie, 1991).

2 MATERIALS AND METHOD

This project was carried out at Al-Ain city. Al-Ain city is located in the eastern province of Abu-Dhabi Emirate, in the UAE. The population consists of nationals and a heterogeneous expatriate population from different parts of the world. The most recent estimated population of Al-Ain, according to the Ministry of Health Annual Report, (1992) is 270.800 (male = 162.900 and female = 107.900) .

2.1 Sources of Material for Study

2.1.1 Hospital Environmental Study:

Al Ain has three hospitals serving the above population. The hospital environmental study was conducted at Al-Ain Hospital which is the largest (511 bed) institution with 23 wards that cover all standard inpatient and several out-patient facilities. It is 14 years old and serves both the national and expatriate resident population of Al-Ain Medical District and is located at the center of Al-Ain city.

The study samples were collected from the following hospital units:

- Male surgical ward
- Female surgical ward
- Male medical ward
- Female medical ward
- Pediatrics ward
- Operating theater
- Intensive care unit

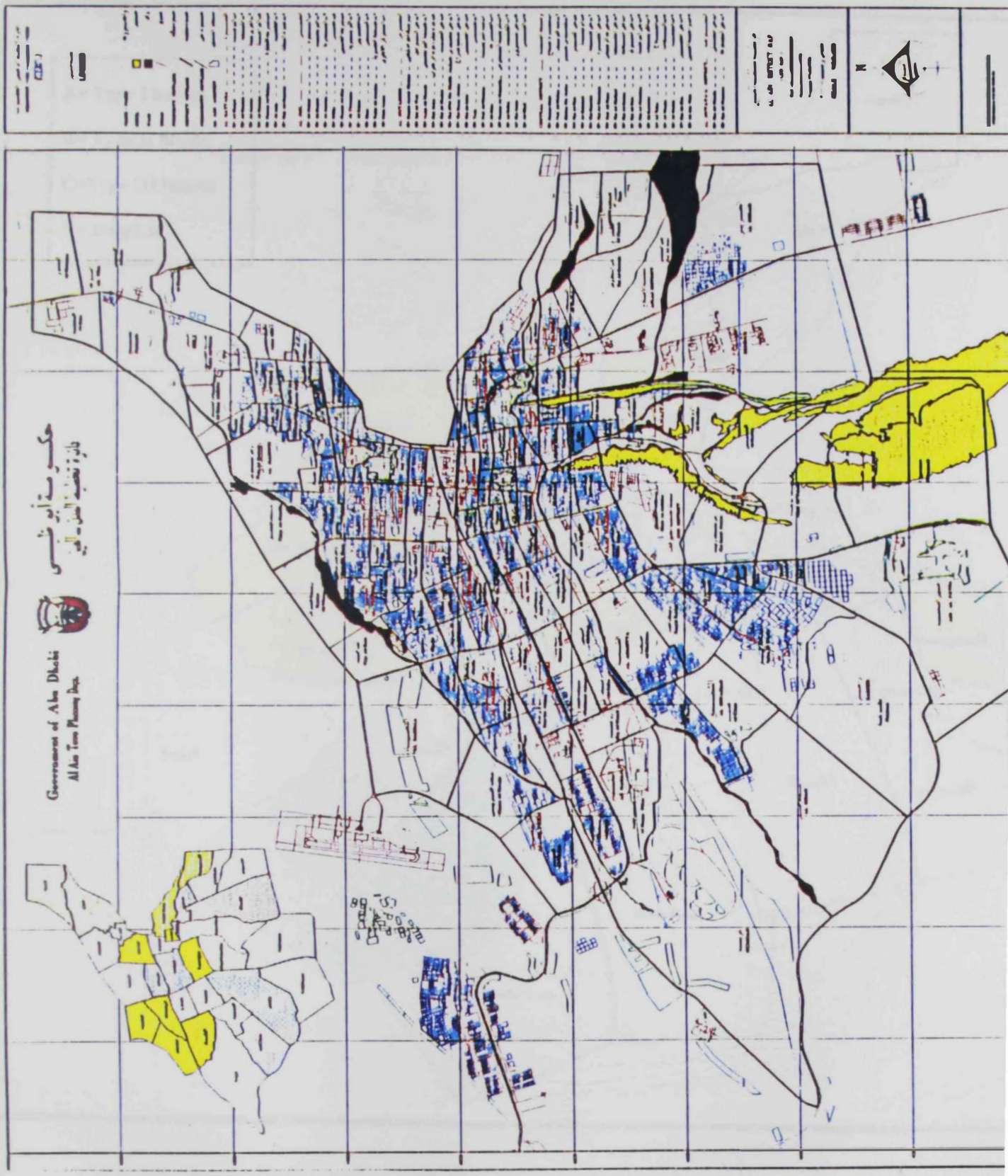


Figure 1- A Map of Al-Ain City

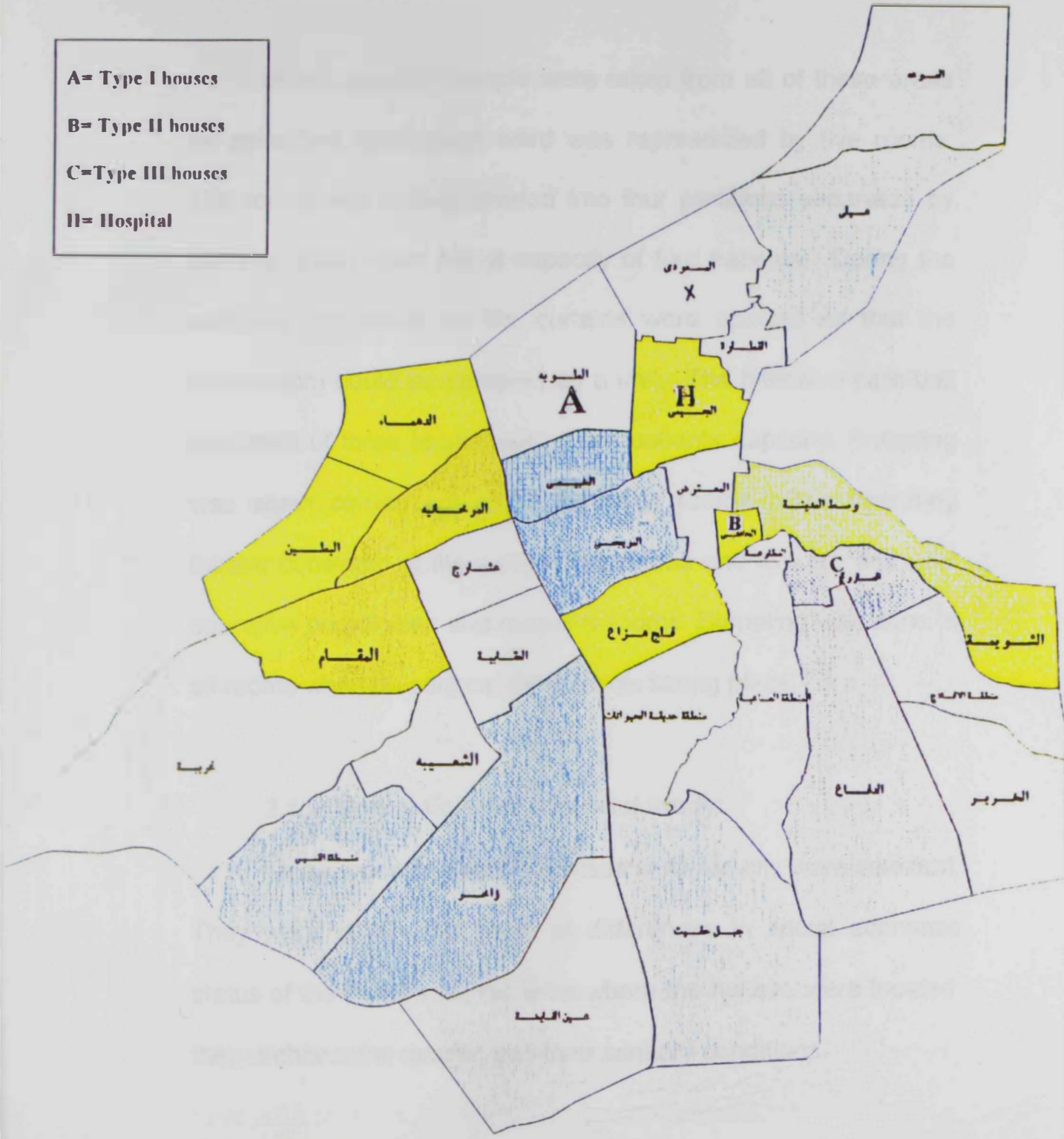


Figure 2 Map of Al-Ain city showing tha location of Al-Ain Hospital and location of homes studed

Microbiological sample were taken from all of these areas as described later. Each ward was represented by five rooms. The rooms are usually divided into four partitions separated by curtains. Each room has a capacity of four patients. During the sampling procedure all the curtains were opened so that the whole room could be sampled as a unit. The intensive care unit consisted of three rooms, with three patients capacity. Sampling was again carried out in all the three rooms. The operating theater consisted of three operation rooms and two pre and post operative preparation and recovery rooms. Sampling was done in all rooms when no surgical activity was taking place.

2.1.2 Domestic environmental study:

Three types of dwelling houses in Al-Ain city were selected. They were graded on basis of differences in social economic status of the occupants; the area where the houses were located, their architectural design, and their sanitary conditions.

Houses were classified as follows:

Type 1 = Very good quality houses

Type 2 = Average quality houses

Type 3 = Poor quality houses

Type 1 Consisted of very spacious newly built and well designed villas. They were located in the up market area of the city. All residents had high income and social strata (according to local grading).

Type 2 were homes built according to the traditional local design, they were built by the government for nationals of medium income. These residances are in groups which are very close to each other and are traditional folk areas.

Type 3 represent poor houses bordering on slums. Each was a single room or more but did not exceed three rooms all which were built at random. These shelters are inhabited by the poor low income expatriate population usually at more than three individals per room.

Figure 3 Photograph of Al-Ain Hospital

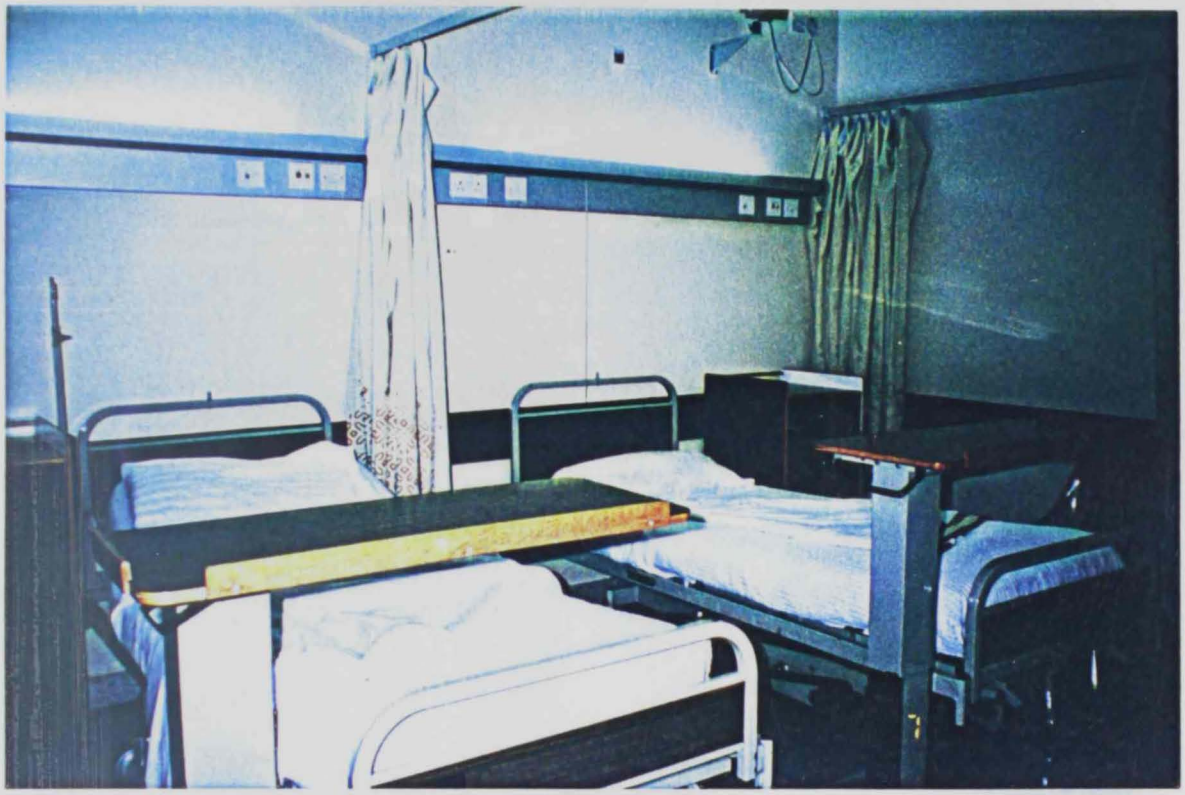


Figure 4 Photograph representing a ward-room inside the hospital

Figure 5 Outside views of a very good house (type 1)

Figure 6 Inside views of a very good house (type 1)

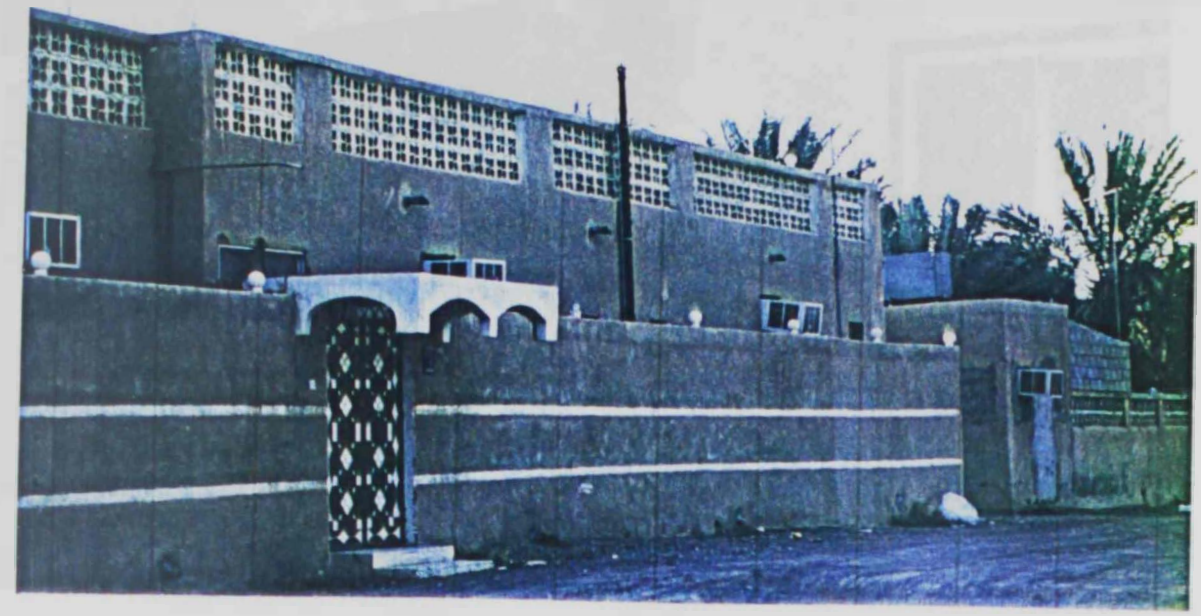


Figure 7 Outside views of a medium quality house (type 2)

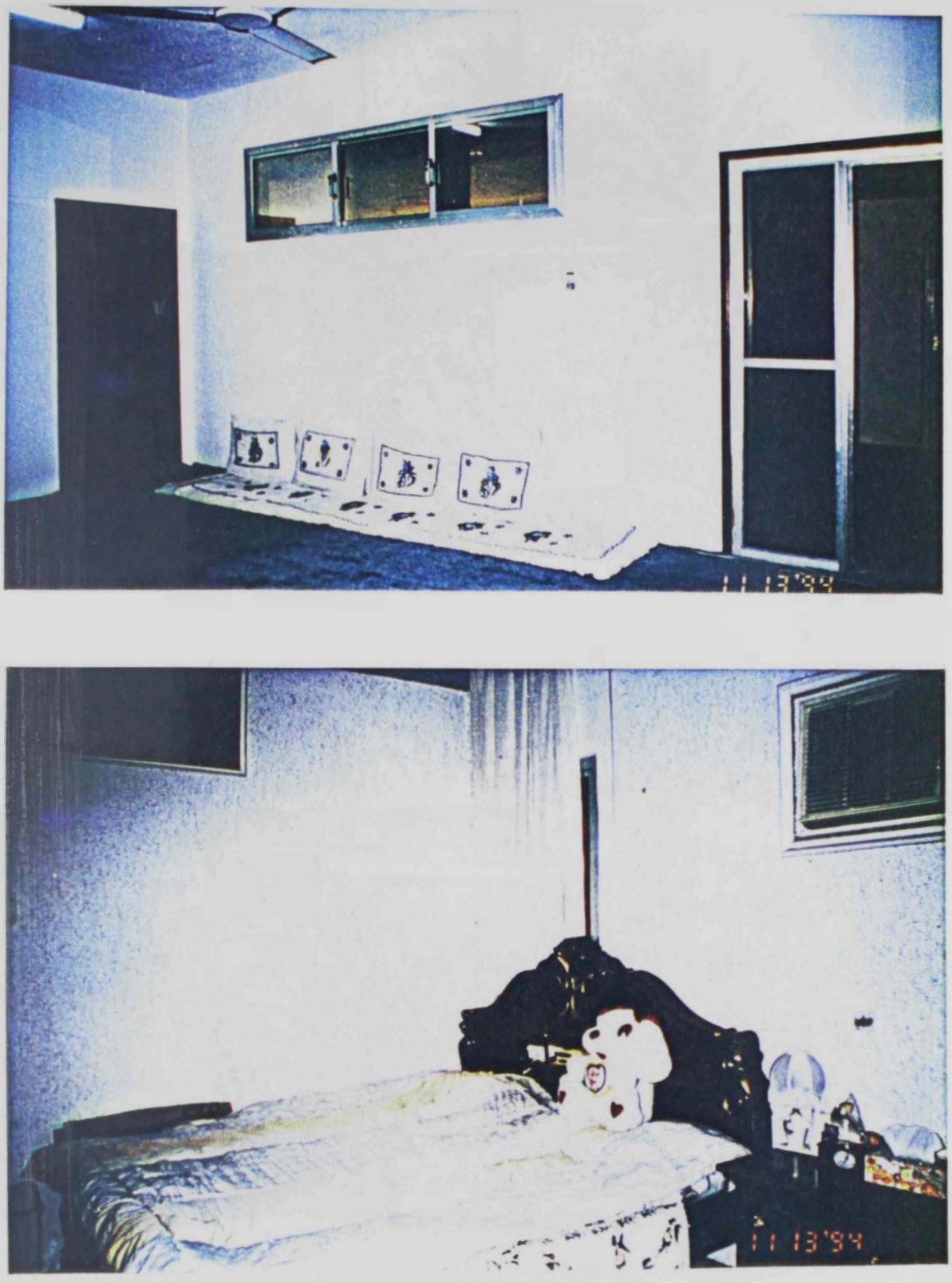


Figure 8 Inside views of a medium quality house (type 2)

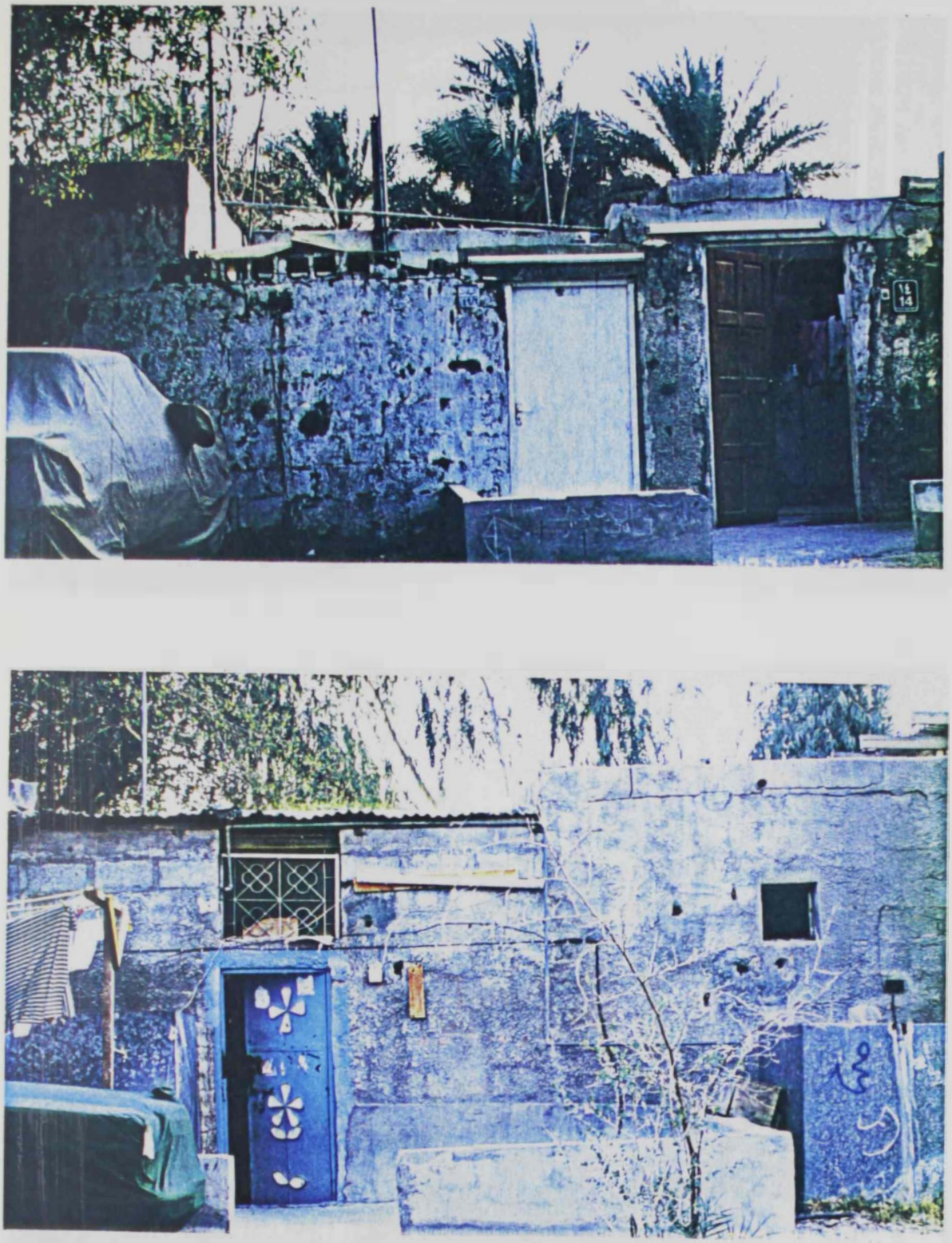


Figure 9 Outside views of a poor quality house (type 3)

Figure 10 Inside views of a poor quality house (type 3)

2.2 SAMPLING TECHNIQUES

2.2.1 Air Samples

Two air sampling techniques were compared for use in this study. These were direct open agar plate exposure technique and bacteria mechanical air sampler, MK2 (Casella London).

a Plate Exposure Technique

This method allows airborne micro-organisms to be deposited on to blood agar plates exposed to room air for definite periods of time. Plates were located in the middle of the room away from opened windows or doors, and about 1 meter above the floor. After that the covers are replaced and plates were then incubated at 37^{o}C for 48 hours after which the colony forming units (CFU) were counted.

b Determination of optimum plate exposure time

Three blood agar plates were exposed in the center of the room, open and facing upwards. The first plate was exposed for 5 minutes and closed, the second plate running

along with the first was exposed for 30 minutes and the third plate was exposed for as long as 60 minutes. This procedure was repeated in five different rooms. All the plates were incubated aerobically at 37^{o}C for 48 hours and CFU were counted and identified.

Table 1 shows that the mean count of CFU per room increases with time of exposure. At 30 minutes exposure the number of CFU was 6 folds higher than at 5 minutes. This was proportional to the time of exposure indicating approximately 2 CFU/min. Longer exposures up to 60 minutes did not produce significant difference. Therefore 30 minutes exposure time was selected for further studies.

c Mechanical Bacterial Air Sampler MK2

The original instrument used was developed by Drs. Bourdillon Lidwell and Thomas of the Medical Research Council (Instruction leaflet 3109/79 bacteria sampler 2).

The air being sampled is drawn at high speed through a narrow slit at a controlled rate; the plate is 0.2 cm below the slit. The surface of the agar plate collects the airborne bacteria and fungi which, because of the high velocity, are caught on the moist agar surface. During sampling, the plate is rotated under the slit to evenly distribute the colonies. The blood agar plate had an inside diameter of 8.5 cm and outside diameter 9 cm.

The machine can be set for three different times: 30 seconds which represents 15 litres of air, 2 minutes representing 60 litres of air, and 5 minutes representing 150 litres of air. To determine the optimum time for the air samples, a comparison of the total number of CFU collected at these three different times in the same room was recorded. It was repeated in five different rooms in the hospital. For each sample the machine was located in the center of the room away from opened windows and doors, and sampling position was 1 metre from the floor.

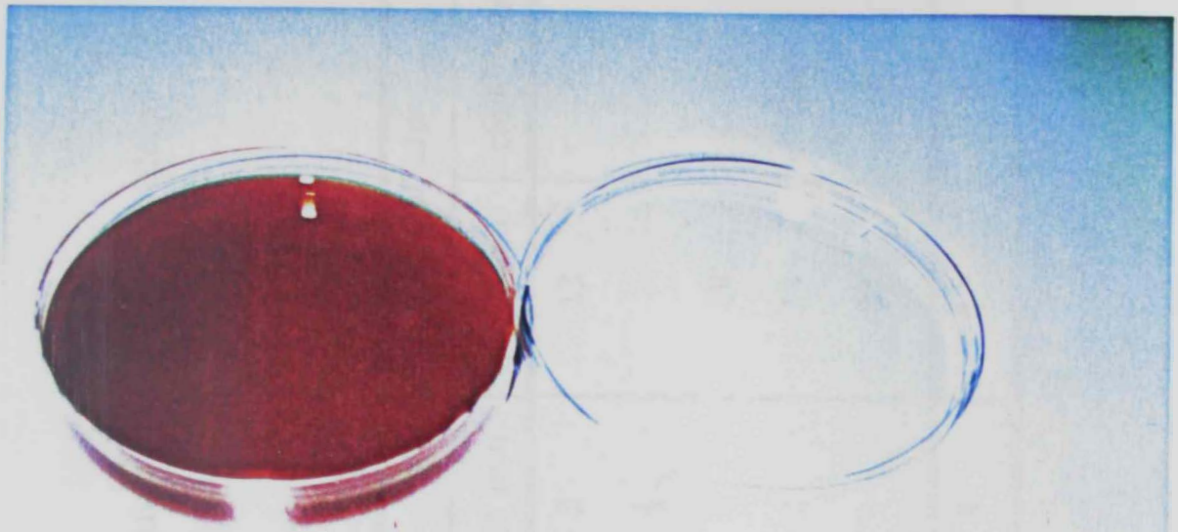


Figure 11 Plate exposure culture method.

Table 1 The total number of cfu on Blood Ager at Different time using plate Air Exposure method

ROOM NUMBER	Total number of cfu		
	cfu/5 min	cfu/30 min	cfu/60 min
Room 1	5	13	15
Room 2	1	7	9
Room 3	6	29	39
Room 4	2	13	14
Room 5	1	23	33
Mean	**3**	**17**	**22**

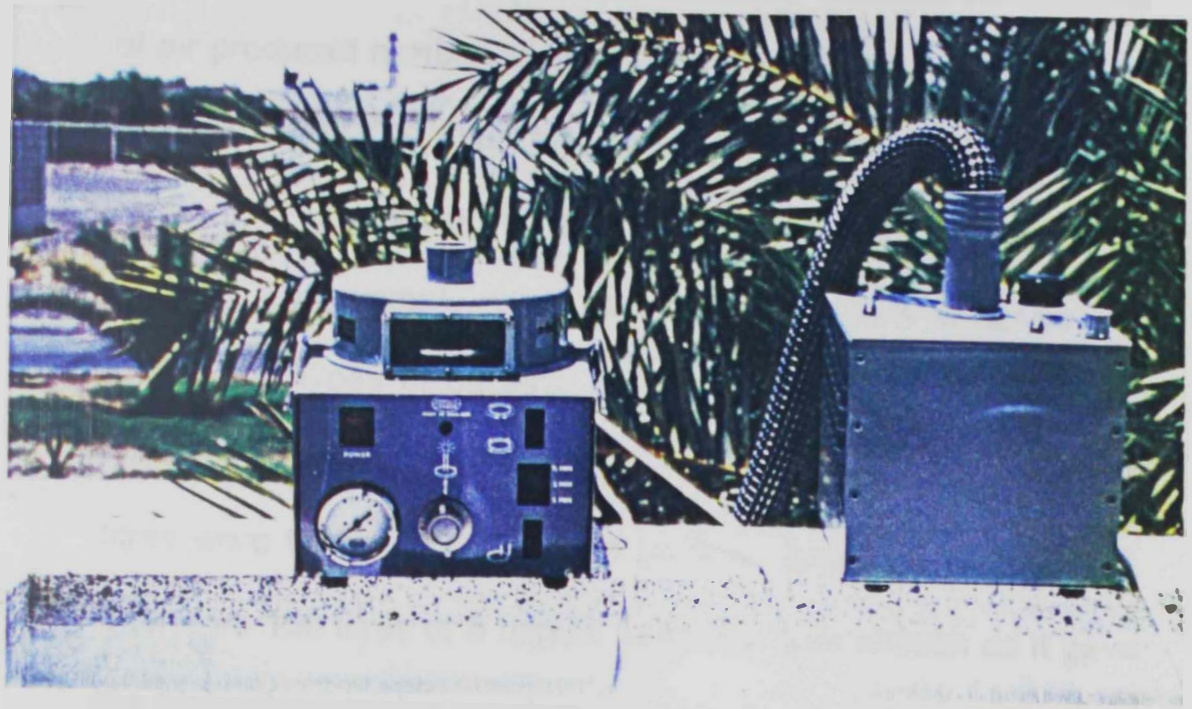


Figure 12 Mechanical air sampler (Casella Bacterial Air Sampler MK2)

d Determination of Optimum Time Required for Mechanical Air Sampler

Table 2 shows the result of different sampling time using the machine air sampler for different rooms. It shows that 15 litres of air produced a mean of 89 CFU, 60 litres produced a mean of 359 CFU, and 150 litres had a mean count of 755 CFU. The types of CFU collected increased with the rise of amount of air sucked. For example, when 15 litres of air were sampled only one or two types of bacteria groups were isolated but when 60 litres were sampled, five to six types were isolated, and when 150 litres were taken, eight types of bacterial groups were isolated. Therefore 150 litres or 5 minute sampling was chosen as it gave the highest countable numbers and larger selection of microbial isolates.

Table 3 shows a comparison between the plate exposure method and the mechanical bacteria air sampler. It shows that the differences between the two methods is only in the number of colonies collected by each method and not in the types of micro-organisms identified. Plate exposure method collected a mean of

50 colonies per 5 minutes, while the machine collected a mean of 4751 colonies per 0.15 m^3 / five minutes, equivalent to 1000 CFU per m^3 in five minutes, in the hospital environment. That reflects the efficiency of the machine which collected amount of colonies easily converted to space and time and taking less time compared with plate exposure method.

Table 2 The total number of cfu on Blood Ager at Different time using Machanical bacterial air sampler

ROOM NUMBER	cfu/30 sec (≈ 15L Air)	cfu/2 min (≈ 60L Air)	cfu/5 min (≈ 150L Air)
Room 1	160	347	760
Room 2	60	293	840
Room 3	173	987	1707
Room 4	13	60	193
Room 5	40	107	273
Mean	**89**	**359**	**755**

Table 3 Micro-organisms isolated by exposure method compared with micro-organisms isolated by Air sampler

(Mean counts per hospital ward)

TYPES OF ORGANISMS	Total No. of cfu/30 min exposure	Total No. of cfu/one M^3 using Air sampler
Staphylococcus aureus	< 1	47
Staphylococcus (CNS)	21	1384
Micrococcus species	4	329
Streptococci (alpha haemolytic)	1	15
Diphtheroid bacilli	3	240
Bacillus species	5	823
Streptomyces species	1	96
Unidentified bacteria	14	1817
TOTAL	**50**	**4751**

2.2.2 Surface Sampling

In each room a test area was selected for surface samples. 5cm^2 were selected from large flat nonabsorbent surfaces such as tables or cabinets; two samples were ccollected from each room. A swab was moistered with sterile distilled water and used to swab the selected surface. The swabs were streaked on blood agar plate and incubated for 48 hours at 37^{o}C. The total number of CFU were counted and the types of bacteria were identified (Macher and Huang, 1991).

2.3 Culture Media

The following bacteriological and mycological media were used for isolation and identification of bacterial and fungal micro-organisms:

- Trypticase soy agar base (BBL microbiology system) and sheep blood supplement for bacteriological isolation.
- Sabouraud Dextrose agar (BBL microbiology system) for isolation of moulds and yeasts.
- Sucrose mineral agar (Czpel-DOX) for moulds identification.

- Mannitol salt agar (Oxoid CM85) selective medium for the identification of presumptive pathogenic *staphylococci.*
- Macconkey agar without salt-(MAST diagnostics) selective medium for differentiation of gram negative Bacilli.
- Mueller Hinton Agar for sensitivity test.

The cultures were incubated for 48 hours at 37^{o}C for bacteria and for 7 days at 25^{o}C for fungi. The CFU were enumerated using Gallen Kamp colony counter and numbers were converted to micro-organisms per cubic meter of air.

2.4 Identification Techniques

2.4.1 Bacterial Identification

Bacterial colonies were initially characterized by colonial morphology and microscopic examination of Grams stained smear. Further identification using oxidase, catalase, coagulase, API 20S, API 20 E for Entenobacteriaceae, and API 20 NE for non fermenting Gram negative Bacilli.

Some bacteria were identified according to Cowan and Steel's (1974) manual for the identification of medical

bacteria, and Identification methods in applied and environmental microbiology (1992), According to all above methods, organisms were classified into nine groups as follows:

1 ***Staphylococcus aureus;*** for all Gram positive cocci, that were catalase positive and coagulase positive.

2 **Coagulase Negative Staphylococci (CNS);** for all catalase positive, coagulase negative gram positive cocci in clusters.

3 **Micrococcus species;** for all strictly aerobic, catalase positive large Gram positive cocci.

4 **Streptococcus species** (alpha haemolytic); for all alpha haemolytic, catalase negative gram positive cocci in chains.

5 **Diptheroid bacilli;** for all catalase positive, oxidase negative, non-spore forming gram positive rods with basically irregular formation.

6 **Gram negative bacilli:** This group included all Gram negative rods which were either Enterobacteriaca and identified by API 20E, and non fermenting group which were identified by API 20 NE.

7 **Bacillus species:** This groups included all the large spore forming Gram positive rods with several different colony morphological characters (rough, smooth, mucoid).

8 **Streptomyces species** were all the slow growing, rough, tough colonies adherent to the medium with earthy odour. Gram stain showed gram positive filaments bacteria.

9 **Unidentified bacteria:** This covers all other organisms, not included in any of the group above. These were generally small colonies that were not characteristic of known human associated organisms. The majority were large Gram negative cocci, some gram positive rods and gram negative rods. All these were negative to all identification tests used to screen the other organisms.

2.4.2 Fungal identification

Fungal colonies were identified by colony appearance and microscopic examination of the spore and hyphal characteristics on lactophenol cotton blue, scotch tape mounts or slide culture where the tape mounts were inconclusive. These were identified according to the manual of soil fungi (Gillman, 1957), and Soil fungi in qatar and other arab countries (Moubasher, 1993).

2.5 Sensitivity Test

The Disk-plate technique was used. In this method a bacterial colony was dispersed in five milliliters of sterile broth, then a sterile cotton swab was used to inoculate the surface of Mueller Hinton Agar Plate. After that the antibiotics were added. The plate was incubated at 37 ^{0}C for 24 hours.

Sensitivity test were performed using sixty Coagulase negative *staphylococci* obtained from three different sources (hospital environment, domestic environment and

patient's). Patient strains were isolated from skin swabs of hospital patient. All strains were tasted against seven commonly used Antibiotics (Penicillin, Ampicillin, Augmentin, Cephalothin, Erythromycin, Tetracyclin and Gentamicin).

2.6 Data processing, Statistical methods and analysis

The variables were coded, and all the data entered and processed in the Department of Community Medicine, Faculty of Medicine and Health Science at the UAE University. Data entry was done by using DOS-5 editor. The statistical software packages SPSS (Norusis, 1992) and BMDP (BMDP, 1992) were used for performing statistical analysis. Harvard Graphics (HG VER 3.0) package was used for graphing.

Basic statistics, frequency table, one way and two way tabulations were obtained. Tests of significance were performed using the chi-square test for two or more categorical variables. In 2 x 2 tables, the Fisher exact test

sample size was small. The significance of difference between two continuous variables were determined by students t-test. Also, the two ways Anova model was performed to explore the joint effects of variables. Means P-less or equal to 0.05 were considered as the cut-off value for significance.

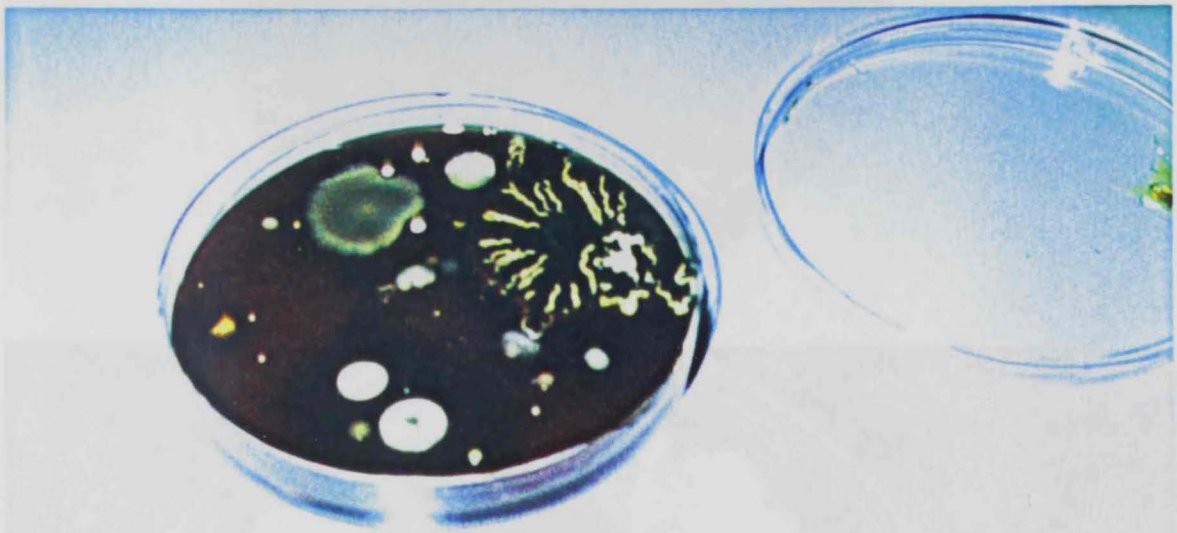


Figure 13 Culture plate after 48 hours incubation time showing bacteria and fungi using plate exposure method for 30 min exposur time.

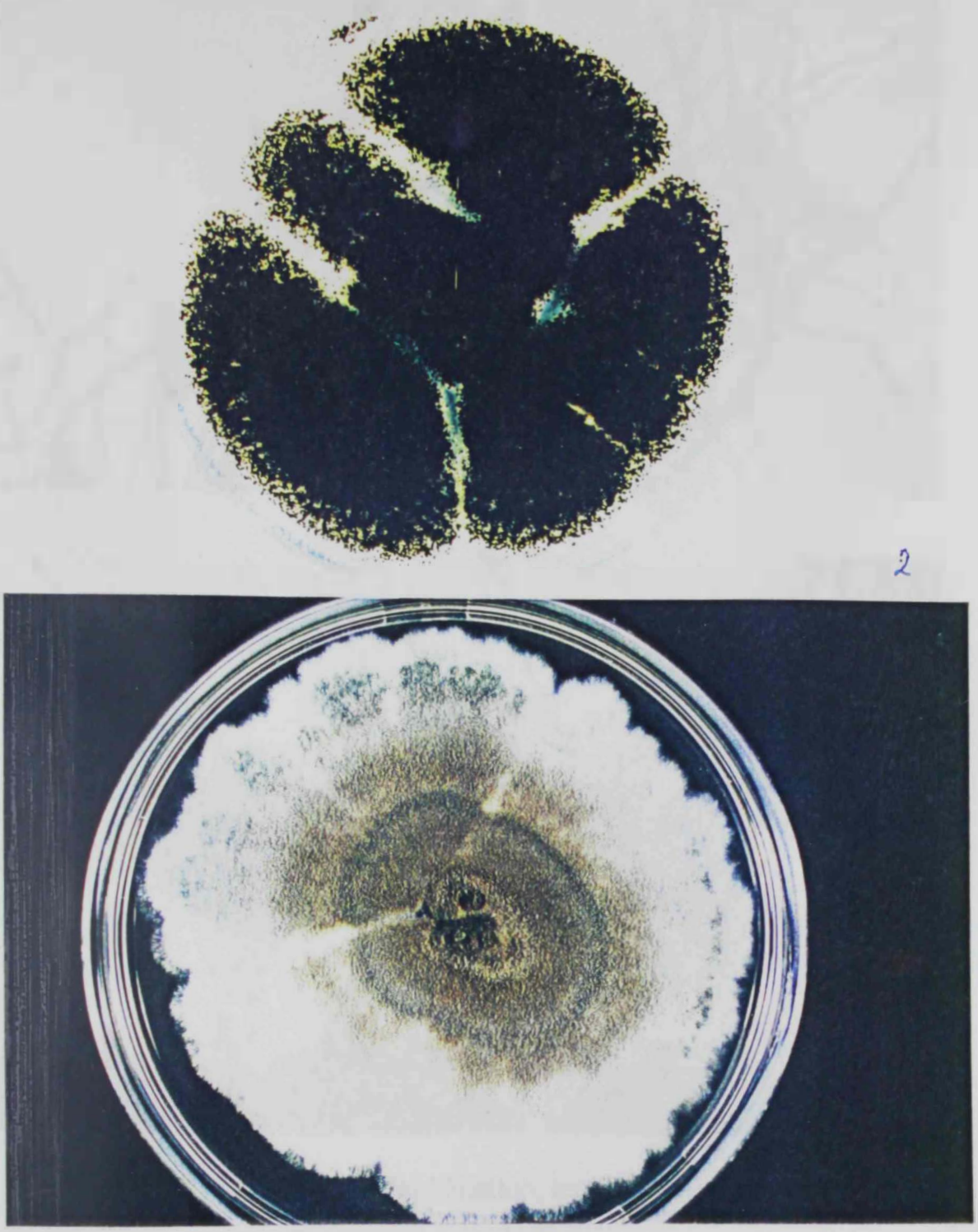


Figure 14 Fungal identification using gross colonial appearance

1. *Aspergillus niger*

2. *Aspergillus fumigatus*

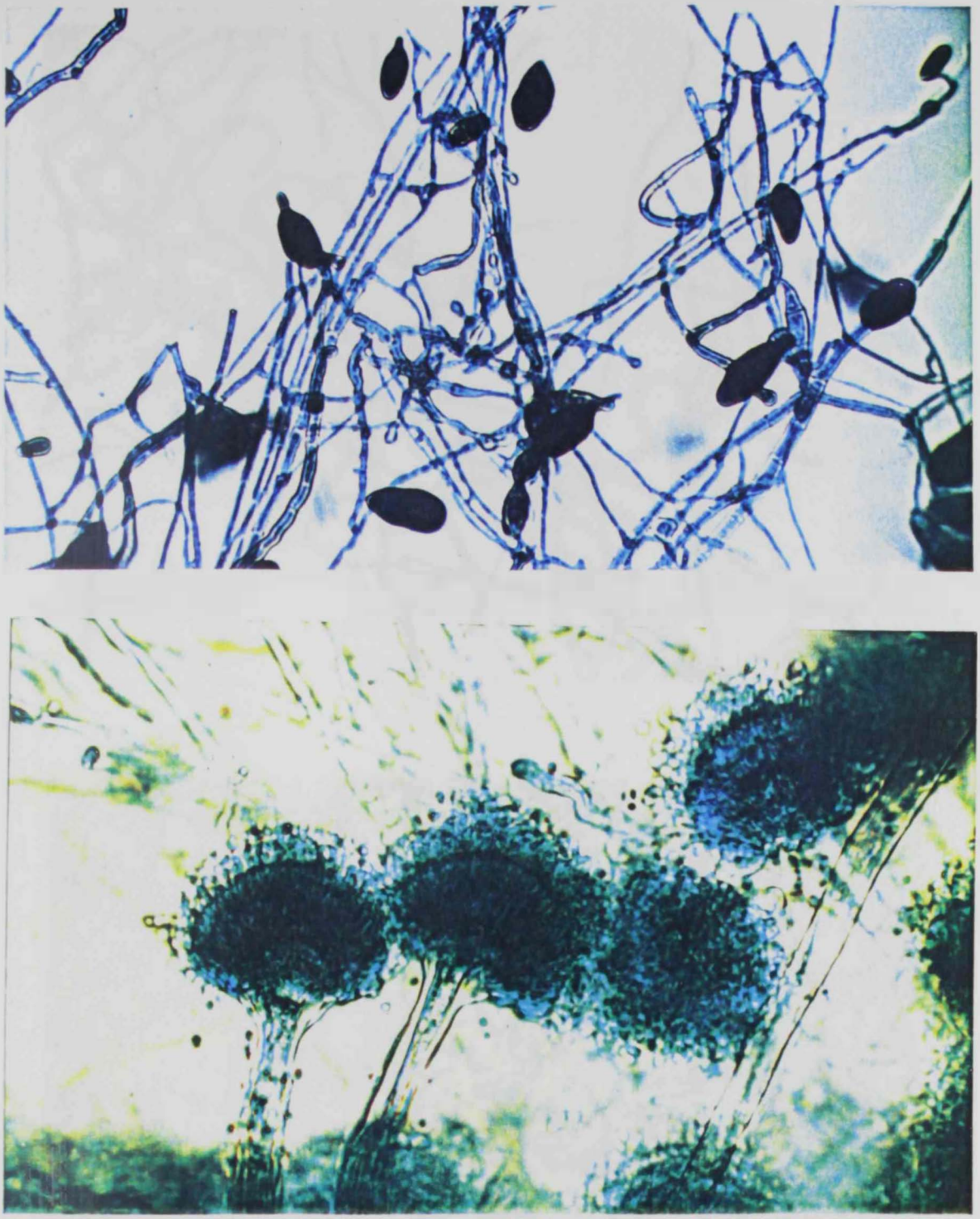


Figure 15 Fungal identification, microscopic examination.

1- *Alternaria species*

2- *Aspergillus niger*

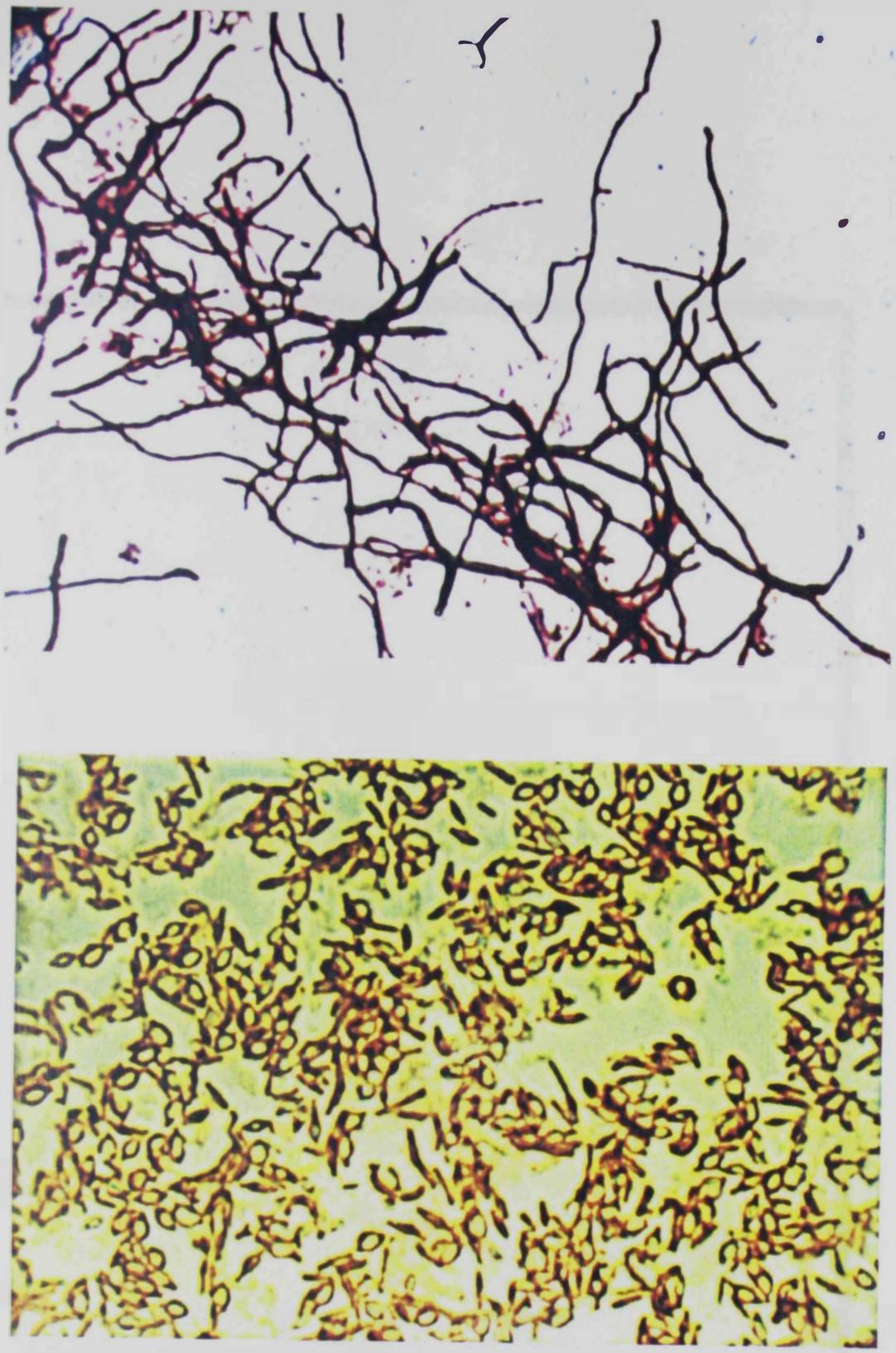


Figure 16 Various unidentified bacteria

CHAPTER III

RESULTS

The main results of the study for the bacterial and fungal counts for both hospital and different homes are shown in tables 4-16 These tables also contain data for surface samples counts.

Table 4 and **figure 17** shows hospital micro-organisms isolated from five different wards in Al Ain hospital. The results reflect the mean of five rooms in each ward. There were nine groups of bacterial in addition to the fungi isolates. These nine groups were divided in to two groups; the human related micro-organisms represented by the first six groups and the environmental and soil micro-organisms represented by three remaining groups.

The most largest quantities of micro-organisms were the unidentified bacteria. This group included different types of bacteria which are not known to be related to medical microbes, difficult to identify, and were possibly soil and airborne bacteria. The second commonest organism occurring in all ward units was

and *Micrococcus species*. All other micro-organisms were detected in small numbers.

It was generally observed that pediatric and female medical wards had the highest bacterial count while the surgical wards had the lowest. The fungal colony counts were close in all wards. In general male wards had less bacterial count compared with female wards.

The classical pathogenic *Staphylococcus aureus* and the Enterobacteriacae group were rarely isolated. *S aureus* was more common in pediatrics and female surgical wards compared to other wards. The gram negative rods which were isolated from pediatric, male medical and female surgical wards included several genera and species such as *Pseudomonas species*, *Enterobacter species*, *Aeromonas species*, and *Esherichia vulneris*. Some of these are known to be occasional human pathogens. The total fungal isolates are included to give a complete picture but will be presented separately later.

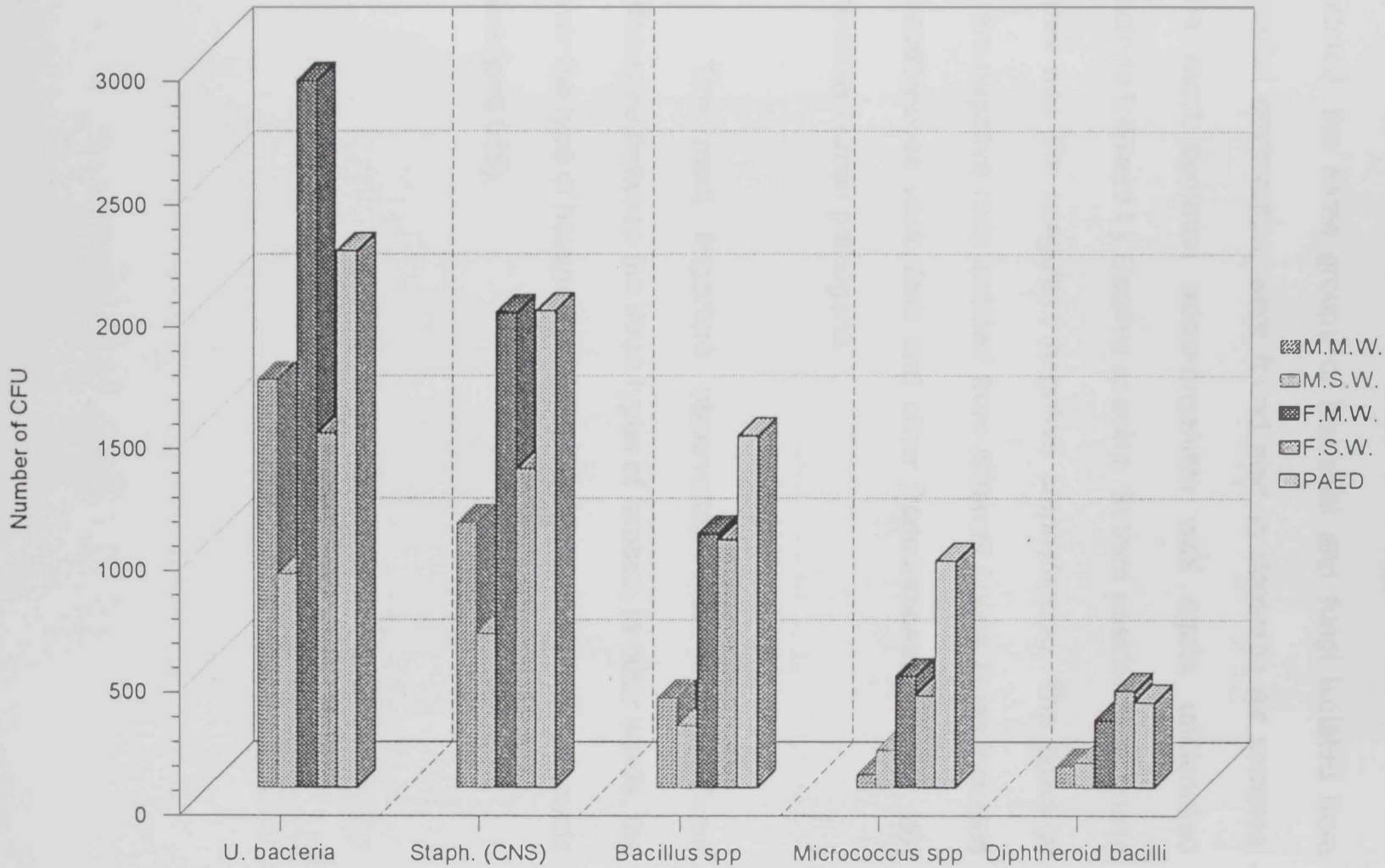
Table 4 Hospital Air micro-organisms isolated from five ward using air sampler

(Figures are means of 5 rooms / ward)

TYPES ●F ●RGANISMS	(No. of cfu/one M^3)					
	M.M.W	M.S.W.	F.M.W.	F.S.W.	PAED	T●TAL
Staphylococcus aures	13	0	0	27	33	73
Staphylococci (CNS)	1087	627	1947	1307	1953	6921
Micrococcus species	53	153	453	373	927	1959
Streptococci (alpha haemolytic)	33	13	0	0	27	73
Diphtheroid bacilli	87	100	273	393	347	1200
Gram negative bacilli	13	0	13	0	60	86
Bacillus species	367	253	1040	1013	1440	4113
Streptomyces species	0	53	220	73	133	479
Unidentified bacteria	1673	873	2887	1453	2200	9086
Fungi	153	133	180	167	73	706
TOTAL	**3479**	**2205**	**7013**	**4806**	**7193**	**24696**

Key: MMW = Male medical ward, MSW = Male surgical ward, FMW = Female medical ward, FSW = Female surgical ward, BAED = Pediatric ward.

Fig (17) Comparing Hospital air micro-organisms isolated from five wards using air sampler



CFU = Colonis Forming Units

U. bacteria = Unidentified bacteria

Table 5 shows micro-organisms isolated from three different types of houses. Except for *S.aureus,* which was not isolated, the same groups of bacterial and fungi isolated from hospital environment were found also in domestic air samples. The most common micro-organism was again, unidentified bacteria followed by *Bacillus species.* In third position in all house types was the coagulase negative *staphylococci.* The types of Gram negative rods isolated from different house types included *Pseudomonas vesicularis* and other Pseudomonas species, not known as human pathogens.

The most important observation was the significant differences between the three types of houses, in other words, the lower the type of houses the higher the number of bacterial counts were ($p<0.045$).

Table 5 Domestic Air micro-organisms isolated from three types of houses

(Figures are mean of 5 houses for each type, values represent cfu/m^3)

TYPES OF ORGANISMS	TPYE OF HOUSES		
	TYPE 1	TYPE 2	TYPE 3
Staphylococci (CNS)	734	2107	3050
Micrococcus species	189	987	2470
Streptococci (alpha haemolytic)	7	74	0
Gram negative bacilli	17	12	120
Diphtheroid bacilli	184	230	4
Bacillus species	1950	2117	3733
Streptomyces species	390	537	893
Unidentified bacteria	2000	3807	4909
Fungi	217	260	273
TOTAL	**5688**	**10131**	**15452**

Table 6 shows the counts and the distribution of micro-organisms in living rooms and bedrooms in the three different types of houses. In the best quality houses (type 1), there were more micro-organisms in bedrooms than in living rooms for three out of four human associated bacterial agents, the opposite was noted for the non human micro-organisms. In type 2 (middle class) houses and type 3 (poor class) coagulase negative *Staphylococci* counts were higher in bed rooms than living rooms while the counts of Micrococci, *Diphtheroid bacilli* and Gram negative bacilli were higher in living rooms than bedrooms. However there was no significant differences between living rooms and bedrooms for any of the organisms isolated. In all types of houses all the environmental (non human related) bacterial counts were higher in living rooms than the bedrooms and the differences between these houses were not significant.

In conclusion the total count of micro-organisms isolated from living rooms were higher than that isolated from bedrooms for all types of organisms except for coagulase negative *Staphylococci* and alpha hemolytic streptococci, which were

higher in bedrooms in all types of houses. There were significant differences between human micro-organisms and environmental micro-organisms in each room in the three different types of houses ($p<0.005$).

Table 6 Domestic air micro-organisms isolated from three types of house comparing living rooms to bedrooms

(Figures are mean of 5 houses for each type, values represent cfu/M^3)

ORGANISMS	SOURCE	TPYE OF HOUSES		
		TYPE 1	TYPE 2	TYPE 3
Staphylococci (CNS)	BR LR	800 667	2360 1853	3906 2193
Micrococcus species	BR LR	237 140	560 1413	2353 2587
Streptococci (alpha haemolytic)	BR LR	13 0	80 67	0 0
Gram negative bacilli	BR LR	33 0	27 33	113 4127
Diphtheroid bacilli	BR LR	87 280	113 327	0 7
Bacillus species	BR LR	1440 2460	1367 2867	3560 3906
Streptomyces species	BR LR	360 420	447 627	753 1033
Unidentified bacteria	BR LR	1987 2013	3460 4153	4380 5437

LR = Living Room BR = Bed Room

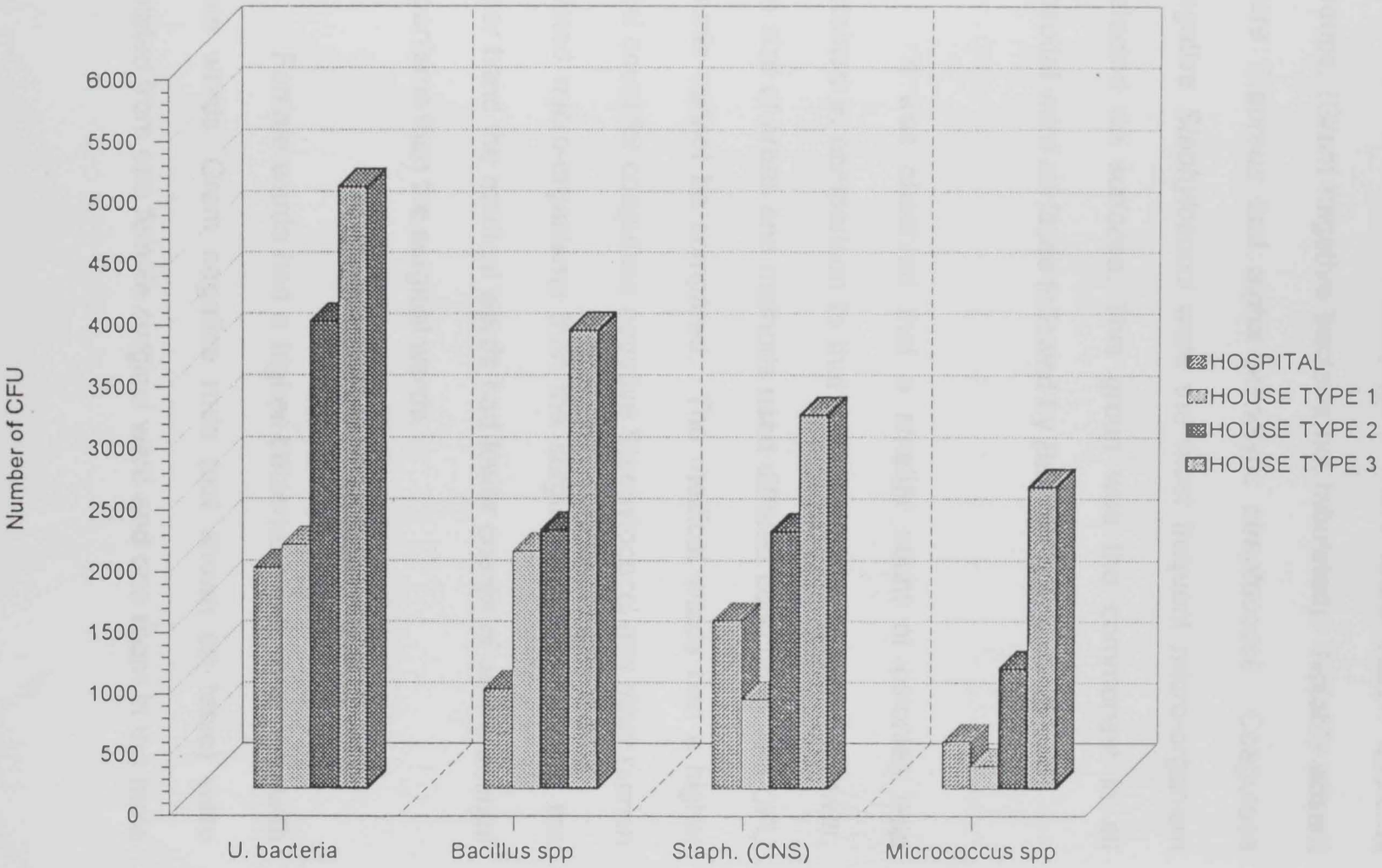
Table 7 and figure 18 presents the numbers of six commonly encountered bacterial and fungal isolates for hospital air and the three different types of house. The total bacterial count of hospital air micro-organisms was the lowest. It was significantly lower than type 2 and type 3 houses ($p<0.05$). Coagulase negative *Staphylococci* and *Micrococcus* species were significantly higher in the hospital environment compared to type 1 houses only. For the environmental bacteria and fungi, the hospital had consistently lower counts and the number increased with the lowing of housing standards. The greatest disparity occurred for all micro-organisms between hospital and type 3 house. The hospital had the lower counts of all types of micro-organisms except for *Diphtheroid bacilli.* The most common group of micro-organisms were the unidentified bacteria. In conclusion the hospital air microbial counts are comparable to house type 1 houses, representing very good quality houses.

Table 7 Micro-organisms isolated from hospital environment compared to micro-organisms from the house environment collected using air samples

(Figures are mean of the number of cfu/one M^3)

ORGANISMS	HOSPITAL	HOUSE TYPE 1	HOUSE TYPE 2	HOUSE TYPE 3
Staphylococci (CNS)	1384	734	2107	3050
Micrococcus species	392	189	987	2470
Diphtheroid bacilli	240	184	230	4
Bacillus species	823	1950	2117	3733
Streptomyces species	96	390	537	893
Unidentified bacteria	1817	2000	3807	4909
Fungi	141	217	260	273
TOTAL	**4893**	**5664**	**10045**	**15332**

Fig (18) Comparing micro-organisms isolated from hospital air with three types of houses



CFU = Colonis Forming Units

U. bacteria = Unidentified bacteria

Table 8 shows the **surfaces** micro-organisms isolated from different wards in the hospital. There were seven bacterial groups, (Gram negative bacteria not tabulated). Notably absent were *S.aureus* and alpha hemolytic *streptococci.* Coagulase negative *Staphylococci* were the most frequent micro-organism detected on surfaces. This group was the commonest in all hospital ward surfaces followed by *Bacillus species.*

It was observed that a smaller count of colonies was obtained in comparison to that from the air samples. However, the size of areas and methods used differed completely and CFU counts cannot be compared. The medical wards had a higher total count for coagulase negative Staphylococci and other human related micro-organisms than the surgical counterparts. On the other hand the medical wards had lower counts of environmental organisms than the surgical wards.

Female wards had a higher bacterial count compared with male wards. Gram negative rods (not shown on table) were isolated from one female surgical ward and one room in the male

medical ward in such a high count that the particular site sampled was considered recently contaminated. These contaminations included, types of known to cause human infections such as *Acinetobacter*, *Klebsiella pneumonia, Pseudomonas stutzeri* and *Pseudomonas putida*. A very high count of alpha hemolytic streptococcus was obtained in one room only in male surgical ward. The differences between human micro-organisms and environmental micro-organisms were significant in female medical and female surgical wards ($p<0.05$), and not significant in male surgical, male medical wards, and pediatrics wards.

Table 8 Surface micro-organisms isolated from different wards in Al Ain Hospital

(Figures are mean of 5 rooms in each ward)

TYPES OF ORGANISMS	(No. of cfu/10 cm^2 surface area)					
	M.M.W	M.S.W.	F.M.W.	F.S.W.	PAED	TOTAL
Staphylococcus (CNS)	56	19	63	20	30	188
Micrococcus species	1	1	3	5	4	14
Diphtheroid bacilli	13	3	5	5	5	31
Bacillus species	7	3	6	15	20	51
Streptomyces species	0	1	0	0	2	3
Unidentified bacteria	3	13	4	5	8	33
TOTAL	**80**	**40**	**81**	**50**	**69**	**320**

Key: MMW = Male medical ward, MSW = Male surgical ward, FMW = Female medical ward, FSW = Female surgical ward, BAED = Pediatric ward.

Table 9 shows the results of counts and types of **surface micro-organisms** isolated from different houses. The numbers of micro-organisms increased with the decreasing status of house. The commonest organism in all house types were *Bacillus* species followed by coagulase negative *Staphylococcus*; *Streptomyces* and unidentified bacteria respectively. All other organisms were isolated in small quantities.

Table 10 and figure **19** compare surface micro-organisms isolated from the hospital and the three different types of houses. The hospital had the highest count of coagulase negative *Staphylococcus* and *Diphtheroid bacilli*, and the lowest count of all other types of organisms. Type 3 houses had the highest count of environmental organisms (*Bacillus species*, *Streptomyces* and unidentified bacteria). Although overall surface count of bacteria in hospital is lower, there is no significant difference with any type of houses.

Table 9 Micro-organisms isolated from surfaces in different house types

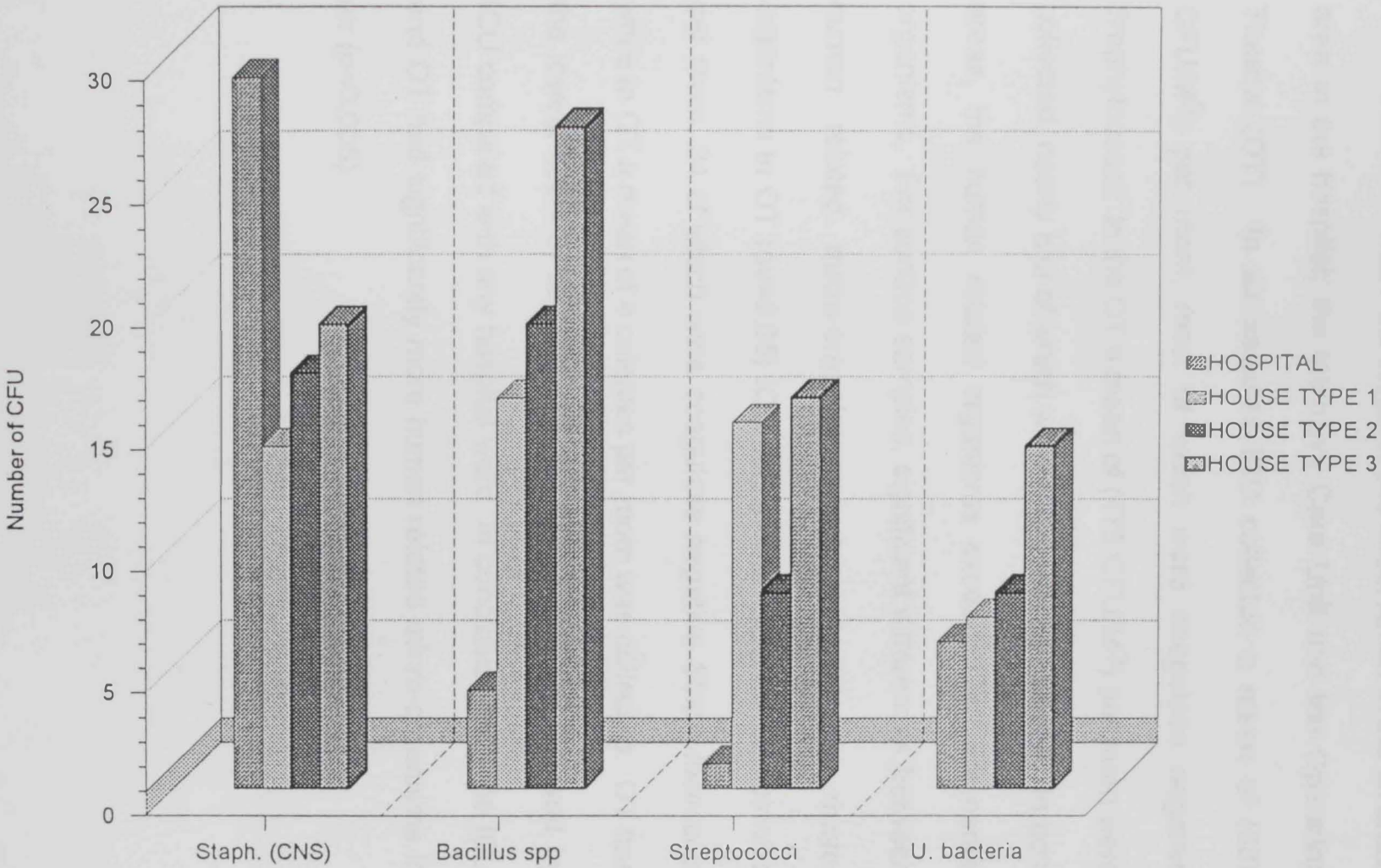
(Figures are mean of 5 houses for each type)

TYPES OF ORGANISMS	TPYE OF HOUSES (No. of cfu/cm^2)		
	TYPE 1	TYPE 2	TYPE 3
Staphylococcus aureus	1	0	0
Staphylococcus (CNS)	14	17	19
Micrococcus species	2	8	3
Streptococci (alpha haemolytic)	15	8	16
Diphtheroid bacilli	0	0	6
Bacillus species	16	19	27
Streptomyces species	2	1	5
Unidentified bacteria	7	8	14
TOTAL	**57**	**61**	**90**

Table 10 Micro-organisms isolated from hospital surfaces compared with those from different house types

TYPES OF ORGANISMS	(No. of cfu/10 cm^2 surface area)			
	HOSPITAL	HOUSE TYPE 1	HOUSE TYPE 2	HOUSE TYPE 3
Staphylococcus (CNS)	29	14	17	19
Micrococcus species	1	2	8	3
Streptococci (alpha haemolytic)	1	15	8	16
Diphtheroid bacilli	2	0	0	< 1
Bacillus species	4	16	19	27
Streptomyces species	< 1	2	1	5
Unidentified bacteria	6	7	8	14
TOTAL	**43**	**56**	**61**	**90**

Fig (19) Comparing micro-organisms isolated from hospital surfaces with those from different houses surfaces



CFU = Colonis Forming Units

U. bacteria = Unidentified bacteria

Table 11 shows the result of the observation in the cleanest area in the hospital; the Intensive Care Unit and the Operating Theater (OT). In air samples ICU collected a mean of (687 CFU/M^3) per room, most of which were coagulase negative Staphylococci. In the OT a mean of (473 CFU/M^3) per room were collected, nearly half of which were *Micrococcus species.* In both areas, the human related organisms exceeded environmental organisms. For *surface* samples, significant differences between human related micro-organisms and environmental micro-organisms in OT (pv=0.05) ICU collected a mean of 25 colonies per room, 24 of which were coagulase negative *Staphylococcus,* while in OT a mean of 4 colonies per room was collected. OT has the lowest count of surface and air micro-organisms followed by ICU compared with any hospital ward. In conclusion both the ICU and OT had significantly more human related micro-organisms in air ($p<0.005$).

Table 12 shows a 2x2 comparison figures of air against surface micro-organisms in hospital and the different types of houses. Although the surface organisms were much lower, usually under 1% of the airborne quantity, they were generally proportional to the quantity in the air. This strongly suggests that the surface organisms originate from the air rather han from other sources.

Table 11 Intensive Care Unit and Operating Theater Micro-organisms isolated from air and surface samples

(Figures are mean of 5 rooms in each area)

TYPES OF ORGANISMS	AIR SAMPLES No. of cfu/M^3		SURFACE SAMPLES No. of cfu/10 cm^2	
	ICU	OT	ICU	OT
Staphylococcus (CNS)	520	80	24	1
Micrococcus species	80	200	0	1
Streptococci (alpha haemolytic)	0	7	0	0
Diphtheroid bacilli	0	33	1	0
Bacillus species	53	93	0	1
Streptomyces species	7	47	0	1
Unidentified bacteria	0	13	0	0
Fungi	27	0	0	0
TOTAL	**687**	**473**	**25**	**4**

Key: ICU = Intensive Care Unit, OT = Operating Theater,

Table 12 Total numbers of air (cfu/M^3) and surface (cfu/10 cm^2) micro-organisms isolated from hospital and the three types of houses

TYPES OF ORGANISMS	SOURCE	HOSPITAL	HOUSE TYPE 1	HOUSE TYPE 2	HOUSE TYPE 3
Staphylococcus (CNS)	Air	1384	734	2107	3050
	Surface	29	14	17	19
Micrococcus species	Air	392	189	987	2470
	Surface	1	2	8	3
Bacillus species	Air	823	1950	2117	3733
	Surface	4	16	19	27
Unidentified bacteria	Air	1817	2000	3807	4909
	Surface	6	7	8	14

The numbers are the mean out of 5 isolation in each case.

Table 13 shows the quantity of fungal spores found in hospital air. It is assumed that each fungal colony originated from a single aerial fungal spore. There were five genera of fungi isolated with a predominance of *Aspergillus species*. Six Aspergillus species were isolated. *A. fumigatus* and *A. niger* were the most dominant. There was no significant difference in the total number of isolates from different hospital units.

Table 14 gives the fungal isolates from the different types of homes. There were six genera and seven species of Aspergilli represent approximately 75% of all isolates. *A. niger* was the most common than any other Aspergillus species depending on there total counts as well as the number of there isolation comparing to other genera. There was an increase of fungal isolates with the lowering of housing standards; concerning *A. niger*, Alternaria and chaetomium but not with other fungal species, which were irregularly isolated.

Table 13 The most dominant fungi in hospital air isolated from five ward using air sampler

ORGANISMS	(No. of colonies/M^3 air)							
	M.M.W	M.S.W.	F.M.W.	F.S.W.	PAED	TOTAL	No. of isolation out of *25	O.R
Alternaria species	4	4	0	4	1	13	8	M
Aspergillus species	**16**	**23**	**34**	**37**	**9**	**119**	-	-
A. fumigatus (Fresenius)	9	15	25	0	4	53	17	H
A. niger (Van Tieghem)	1	4	5	16	4	30	10	H
A. sparsus (Thom & Rapper)	1	0	0	4	0	5	2	L
A. tamarii (Kite)	0	0	0	9	0	9	1	L
A. terreus (Thom & Rapper)	4	0	4	7	0	16	8	M
A. versicolor (Tiraboshi)	0	4	0	1	1	6	7	M
Chaetomium species	11	11	5	0	1	27	7	M
Penicillium species	1	0	9	0	0	10	4	L
Verticillum species	0	0	0	4	0	4	2	L
TOTAL	**31**	**38**	**48**	**45**	**12**	**173**		

Key: MMW = Male medical ward,
MSW = Male surgical ward,
FMW = Female medical ward,
FSW = Female surgical ward,
BAED = Pediatric ward.

*** 25 is the number of isolats**
O.R = Occurrence Remark,
H = High Occurrence more than 10,
M = Moderate Occurrence 5-9,
L = Low Occurrence less than 5.

Table 14 Air-borne fungi isolated from three types of houses

TYPES ●F ●RGANISMS	TPYE ●F HOUSES (No. of colonies/M^3 air)			No. of isolation out of *15	●.R
	TYPE 1	TYPE 2	TYPE 3		
Alternaria species	0	47	127	6	M
Aspergillus species	**225**	**153**	**327**	-	-
A. fumigatus (Fresenius)	0	0	53	3	L
A. flavus (Rapper & Fennel)	33	0	0	2	L
A. niger (Van Tieghem)	80	153	240	15	H
A. sparsus (Thom & Rapper)	33	0	0	1	L
A. terreus (Thom & Rapper)	33	0	0	5	M
A. tamarii (Kite)	46	0	7	2	L
A. versicolor (Tiraboshi)	0	0	27	1	L
Chaetomium species	20	53	80	6	M
Mucor species	0	0	7	1	L
Penicillium species	0	113	7	5	M
Trichoderma species	47	0	0	1	L
Verticillum species	13	0	0	1	L
TOTAL	**305**	**366**	**548**		

*** 15 is the number of isolats**

Key: O.R = Occurrence Remark,
H = High Occurrence more than 10,
M = Moderate Occurrence 5-9,
L = Low Occurrence less than 5.

Table 15 comparing Al Ain hospital fungal isolates to those of the 3 types of houses, like bacterial isolates, the hospital had lower total fungal counts compared to the domestic environment. Although there was no significant difference between hospital and type 1 and 2 houses the difference was significant for type 3 house. In total there were seven genera of fungi isolated in the indoor environment of hospital and homes. *A. niger* and *Chaetomium* were the most constantly found in all areas investigated.

Table 16 shows antibiotics sensitivity test for sixty coagulase negatives *staphylococci* (CNS) obtained from three different sources. Twenty were from domestic areas, twenty from hospital environment, and twenty from hospital patients. They were tested against seven commonly used anti microbial agents listed in the table. The results are expressed as a percentage of organisms sensitive to the drug.

The domestic CNS were highly sensitive to the seven drugs (mean sensitivity of 93%) and completely sensitive (100%) to five of the drugs. The hospital environmental CNS were 100% sensitive to gentamicin only and moderately sensitive to five drugs but generally resistant to erythromycin.

In contrast to the CNS, isolates from patients' specimen were evaluated simultaneously. These strains were less sensitive than even the hospital environmental organisms except for erythromycin and tetracycline. There was a very significant difference between sensitivity of patients' strains and hospital strains for penicillin and ampicillin but not for augmentin. There was a moderate difference with cephalothin and gentamicin.

The overall analysis shows that the resistance of hospital environmental strains were in between the domestic and the patients' strains.

Table 15 Air Fungi isolated from hospital and three types of houses

TYPES OF ORGANISMS	(No. of colonies/M^3 air)			
	HOSPITAL	HOUSE TYPE 1	HOUSE TYPE 2	HOUSE TYPE 3
Alternaria species	13	0	47	127
Aspergillus species	**119**	**225**	**153**	**327**
A. niger (Van Tieghem)	30	80	153	240
A. fumigatus (Fresenius)	53	0	0	53
A. tamarii (Kite)	9	46	0	7
A. terreus (Thom & Rapper)	16	7	0	0
A. sparsus (Thom & Rapper)	5	33	0	0
A. versicolor (Tiraboshi)	7	46	0	27
Chaetomium species	7	20	53	80
Penicillium species	4	0	113	7
Mucor species	-	0	0	7
Trichoderma species	-	47	0	0
Verticillum species	2	13	0	0
TOTAL	**173**	**305**	**366**	**548**

The numbers are the mean out of 5 isolation in each case.

Table 16 SENSITIVITY PATTERN OF COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATED FROM DIFFERENT AREAS AND PATIENTS

Source of CNS	Pen.	Amp.	Aug.	Cep.	Ery.	Tet.	Gen.	Mean
Domestic	100	100	100	100	55	95	100	93
Hospital	75	80	85	95	35	60	100	75
Patients	20	42	82	82	50	60	89	60
Mean	**65**	**74**	**89**	**92**	**46**	**72**	**96**	**76**

Key: Pen = Penicillin, Amp = Ampicillin, Aug = Augmentin, Cep = Cephalothin, Ery = Erythromycin, Tet = Tetracyclice, Gen = Gentamicin.

CHAPTER IV

DISCUSSION

Indoor air contains a large microbial population. The significance of these microbes is debatable in some quarters, whereas elsewhere it is considered significant. The importance of the estimation of the numbers and types of airborne micro-organisms is that it might be used as an index for the cleanliness of the environment (William *et al.*, 1956).

Although work elsewhere has shown what is expected in hospitals, there is very little in the literature on domestic microbial flora. This study therefore is new as far as this area is concerned, particularly so, in the Gulf States.

In order to evaluate the quality of the air in the hospital, and to find the relationship between the bacterial count and the environmental condition, a comparison between the hospital and the three types of houses were carried out.

Airborne infections particularly of the respiratory nature in domestic areas and in crowded conditions are extremely common. Airborne spread of nosocomial bacterial or viral infection is known to occur, but is probably uncommon, therefore air sampling should be carried out less frequently. Perhaps the most common and recent indications for use of this technique have involved *Legionella* and *Asperigillus* infections and in monitoring of operating room infections.

Air sampling may be performed with either settling plates or by using more sophisticated equipment. Plate exposure method is one of the simplest methods used for air examination. It has been recommended for the quantitative evaluation of airborne bacteria, however, it collects mainly those particles large enough to be pulled by gravity or impacted by air turbulence onto the collecting surface (Herman and Morelli, 1961; Wolf *et al.*, 1964). For these reasons airborne particles that are too small to settle out quickly may not be collected. Additionally if the turbulence of the air to be sampled varies from time to time, the yields of the exposure plate will also vary (Cole and Bernard, 1962). Although

this is an inexpensive way to evaluate airborne microbial contamination, quantitative results may correlate poorly with those obtained with mechanical volumetric air samplers. Because of variation in particle size and unknown influences of air turbulence, nuclei of approximately three micro meter can remain suspended indefinitely and can only be collected with high-velocity, volumetric air samplers.

Although Sayer *et al.* (1972) have reported that Gravity Settling Culture (GSC) Plate should not be used for quantitative estimation of hospital airborne bacterial flora, plate exposure method was used in this study to evaluate the efficiency of the bacterial air sampler which was being used for the first time in the Al-Ain study. The comparison between the two methods shown in table three shows that plate exposure method for 30 minutes collected a total of 50 CFU against 4750 collected by the mechanical air sampler in five minutes: this is just over one percent efficiency as far as quantity is concerned. Furthermore qualitatively the same types of micro-organisms were collected by the machine in five minutes sampling time as by plate exposure

method in 30 minutes. We can quantitate the micro-organisms of the bacterial air sampler as 0.15 m^3 per 5 minutes whereas the plate exposure method cannot be estimated in terms of air volume. For this reason the superior mechanical air sampler (Casella bacteria sampler MK2) was chosen. Although there are several types which are more convenient, we used Casella bacteria sampler MK2 which was available to us.

Sayer *et al. (*1972) reported that airborne *Staphylococcus aureus* was detected in hospital by the Andersen sampler but not by the accompanying GSC plate. In this study however, the plate exposure method collected only 2 colonies of *S aureus*, and a mean of 21 colonies of coagulase negative *staphylococcus* per room. This finding is in line with Hall's findings (1962), when he considers the GSC plate, generally adequate for collection of *Staphylococci* in suspected hospital rooms.

Walter (1966), has divided hospital airborne bacteria into three groups in descending of the size of particulate:

1) Dust borne bacteria i.e. passengers on rafts of debris, skin scales.

2) Droplet borne bacteria i.e. passengers within fluid droplets.

3) Bacteria representing the droplet nuclei from which most or all of the moisture has evaporated.

The Andersen air sampler which was used by Sayer *et al.* (1972), relates the isolated bacteria to the parent particle size, making it possible to predict the magnitude of inhalation risk. The air sampler used in this study does not do that, therefore the isolated micro-organisms were divided into two groups.

1) Human associated organisms which are normally found in or on human body and clothing, could be generated from human activities and were not isolated from outdoor air samples. These include: *S.aureus,* coagulase negative *Staphylococcus*, *Micrococcus* species, alpha hemolytic *Streptococci*, Gram negative rods, and *Diphthiroids species.*

2) Environmental organisms which come from other sources such as air dust, soil, and water. These were most frequently isolated from outdoor air samples. These

included *Bacillus species, Streptomyces species*, and unidentified bacteria.

Unidentified bacteria were the commonest group of bacteria isolated from either hospital or domestic air samples. These were micro-organisms of no medical importance. They consisted of aerobic gram negative cocci, larger than *Neisseria* and *Moraxella* species. There was also a large presence of very small gram positive rods and gram negative rods. It was concluded that this group was mainly soil borne bacteria which have also been found to be dominant in the dust of three types of dwellings in India (Raza, 1989).

Quantitative study of different hospital units showed that the pediatric ward and female medical wards had the highest total count of bacteria. This could be due to many factors, as discussed by Woods, 1986. These findings could be explained by the fact that the number of visitors in pediatric and female medical wards exceed visitors in other hospital areas. For example in one room in pediatric ward, there were seven children and three ladies

at the same time during sampling process and in one female medical ward room there were five female visitors in only one partition of the four partitions. At no time did the visitors in the male wards exceed three during sampling processing. It was also observed that the amount of materials brought from outside, such as carpets, flowers, fruits, were more common in pediatric and female wards. There may be other environmental and human factors that could have contributed to these inconsistencies, which were not observed.

In comparison to other bacteria the numbers of pathogenic micro-organisms in the hospital air was found to be low. Pathogens represented less than 1% of the total count of bacteria isolated. Using an Andersen air sampler, William (1956) found that 3% of the total count of organisms were pathogenic bacteria. However, he included *Streptomyces* species, as human associated micro-organisms. In this study *streptomyces* species was considered as a environmental micro-organism. The reason for this consideration that the types of *streptomyces* isolated were unlike those that cause actinomycetoma.

The low concentration of pathogenic organisms in hospital air could possibly be due to the fact that there was not enough air current to distribute the bacteria from the reservoir (patient). In order to evaluate this possibility, air drafts were created by aerating and rearranging the patient bed sheets, curtains and carpets if present. Air samples specimens were taken before and after this practice. Samples collected after aerating showed double the numbers of CFU total count as compared with samples before aerating. Both samples showed the same types of micro-organisms which did not include pathogenic ones.

Pathogenic organisms of the type causing wound infections, should theoretically be in high concentration in certain hospital areas but this is not the case as they do not seem to be airborne. The mode of transmission of these is likely to be via physical contact of staff rather than by airborne means. In airborne infections such as throat infections the causative agent (pathogenic *streptococcus*) is usually isolated in a small numbers (i e. 10 to 20 colonies per swab); whereas one could find a 10^6

CFU/swab of non pathogenic *streptococcus* such as *S. viridans* isolated from same throat swab of the infected patient

It was interesting to compare the types of airborne micro-organisms found in hospital to those at the home environment. As this was the main purpose of this study, serious efforts were made to understand the differences, similarities and the relationship of the two environments.

Human pathogenic micro-organisms particularly *S.aureus* and members of Enterobacteriacae were more prevalent in hospitals than in homes. *S.aureus* is not a very common skin or respiratory flora. It would be expected that it come from bacterial lesions or different types of infections in patients. These patients would, therefore, be hospitalized and their micro-organisms spread to some degree. Enteric organisms are likely also to arise from wounds, stools and urine of infected patients. Enterobacteriacae were more common in hospital environment, both for air and surface samples, than in domestic areas as it would be expected.

Although there are no standards for viable or nonviable particulate in the operating room, or in any other hospital area (Kundsin, 1985), the number of micro-organisms in operating rooms and Intensive care unit was very low. This was anticipated due to the high sanitation level present in these areas, as compared to other hospital areas.

The comparison of micro-organisms found in homes and the hospital showed that there were more micro-organisms in residential houses in both air and surfaces than in the Al Ain hospital. The microbial counts found in the highest status houses compared favorably to that of the hospital and therefore, it could be concluded that the these houses have hygienic standard close to that found in the hospital.

On the other hand, there is a noticeable difference between the hospital and types two and three residential microbes. This however reflects in quantity rather than in the types of microbes. The poor quality houses had the higher bacterial population.

Raza (1989) has reported that the bacterial population in the indoor dust of slum dwellings was higher than that in middle class and upper class dwellings. The fact that the microbial flora of the indoor air depended on the number and types of people present (Dugid, 1946), the quality of the household system (Fink *et al.*, 1971; Fraser *et al*,. 1977; Pickering *et al*,. 1976), and mechanical movement within the enclosed space. This fact can explain these findings because in the poor houses there are too many people in a small space. Therefore, whatever microbes are shed would lead to a build up of numbers in this confined space.

In contrast, in the best type of house, there are fewer residents per cubic meter (area) of house space occupied and therefore, shed microbes are spread out in the space available. In addition, the better the house, the better the ventilation to sweeps out microbes from indoor environment.

Cleaning procedures also vary between hospitals and domestic areas and between the different types of domestic environments. It was observed that the best type of house had

many domestic cleaners, good housekeeping procedures and a good air conditioning system that augur well for good indoor standards.

The similarity in the number of micro-organisms in the hospital and in the very good quality homes is an indicator that both areas have a good hygienic standard. In Al-Ain Hospital international standards for sanitation through sterilization, disinffection and cleaning are applied, therefore the hygienic standard of air and surfaces is high. The hospital administration should be congratulated for that.

It is however not surprising that the environmental micro-organisms are found in much higher numbers as the housing standards drop. The effect of desert storms and spread of soil bacteria would be interesting to study.

On visiting home areas, one other significant observation made was the difference in the numbers of human associated and environmental micro-organisms between the living area and

sleeping quarters. It was particularly noticed that the number of human and environmental microbes in living rooms and bedrooms were found to be higher in the lower status homes. Human related microbes were found in bedrooms more frequently than living rooms. The implication is that more time is spent in bedrooms than living rooms. On the other hand the soil, water and air micro-organisms, were found more frequently in living rooms than bedrooms. This indicates probably that bedrooms have less exposure to dust than other rooms, regardless to the status of the house.

Comparison of microbial numbers in air samples and on surface are not considered here. The two systems are not supposed to be comparable but Buttner and Stetzenbach (1993) suggest that they are related in certain circumstances . However, the types of microbial genera (or species)and not the numbers of microbes in these two systems were the same, therefore one would postulate a relationship between the deposit from air to surface by gravity and from surface to air, by currents.

The study of fungal air spora is of great importance and interest in order to understand the dissemination, spread, and movement of the microbes, particularly the pathogenic ones in the atmosphere (Mostafa, 1976).

In this study three genera of fungi were frequently isolated from hospital air and the three types of houses these were *Asperigillus niger,* a *Chaetomium species*, and an *Alternaria species*. Another two *Penicillium species* and *Asperigillus tamarri*, were isolated less frequently. Seven *Asperigillus species* were isolated in this study. Kodama (1986) reported that the air inside airconditioned homes was found to have fewer fungi, but had a significantly greater number of *Asperigillus* species, when compared to the outdoor air. Raza (1989) reported that *A. flavus* and, *A. niger* were dominant in all the types of dwellings in India. Also he observed a high concentration of *Asperigillus* in most indoor environments. It was also found that the most frequent isolates of *Asperigillus* species from outdoor air were *A. flavus*, *A. niger, A. fumigatus and A. terreus* in Kuwait (Mustafa, 1976). This

can explain the high concentration of *Aspergillus* species compared with other fungal groups.

The study of antibiotic sensitivity pattern of patient's micro-organisms is of great clinical relevance and value. It is also appreciated that the knowledge of anti-microbial sensitivity pattern is also of epidemiological value. Microbes from the same source usually exhibit similar susceptibility to the same anti-microbial agents. When the source of a bacterial epidemic disease is investigated, domestic or hospital environmental micro-organisms are compared with the patients' organisms. One of the tools used, among many sophisticated techniques available, is the antibiotic sensitivity comparison. In this study, this relationship has been evaluated using coagulase negative *Staphylococci* (CNS). It was clearly observed that there was a difference in sensitivity patterns for hospital-associated micro-organisms and those isolated from homes. The results also showed that domestic airborne CNS were sensitive to nearly all the antibiotics tested. The source of domestic strains was the established environment or from people who had not recently used any anti-

microbial agents. A few of the domestic strains were resistant to some antibiotics such as tetracycline. This resistance was probably due to the fact that some bacteria are naturally resistant to a particular antibiotic even if they were never exposed to the same drug (Pelczar *et al.*, 1993).

When the environment is stable, the bacterial population will remain unchanged, but if any factors affecting the stabilaty changed to hostile environment, only certain individual microbes bearing protective mutation can adapt and survive. In this way, the environment naturally selects certain mutant strains that will reproduce, give rise to subsequant generations, and in time, be the dominant strain in the population. One of the clearest models for this sort of selection and adaptation is acquired drug resistance in bacteria (Talaro and Talaro, 1993).

When antibiotics were first used in chemotherapy, development of antibiotic-resistant microorganisms was infrequent. However, as antibiotics became widely used, resistance became much more of a problem as susceptible

microbes were eliminated and the numbers of resistant micro-organisms increased. The initial appearance of a resistant bacterium in an otherwise susceptible population is often caused by a mutation in a single bacterial gene. The gene for resistance can be transmitted from a resistant cell to a susceptible cell by conjugation. This process is called transmissible antibiotic resistance. It is however not known whether the environment where this process takes place has any significant effect (Pelczar *et* al,. 1993).

Patients strains were the most resistant ones compared to domestic and hospital strains. Patients strains are usually subjected or exposed to anti-microbial agents used by hospitalized patients. The appearance of drug-resistant bacteria is a reflection of the forces of natural selection because the drug-resistant variant from of pathogens has a unique fitness trait that is absent from the original species, it possesses physiological or structural properties that allow it to grow in the presence of a stress not tolerated by the original population (Boyd, 1988). In ecological terms the environmental factor (in this case, the drugs)

has put selection pressure on the population, thus the fitter microbe (the drug-resistant one) survived, and the population has evolved to a condition of drug resistance. Natural selection for drug-resistant forms is apparently a common phenomenon. It takes place most frequently in various natural habitats, laboratories, and medical environments.

The hospital airborne micro-organisms seem to be a mixture of patients', environmental and visitor's strains as their sensitivity falls between domestic and patient strains.

CHAPTER V

CONCLUSIONS

Human pathogenic micro-organisms particularly *S.aureus* and members of Enterobacteriacae were very infrequent.

Microbial flora of the indoor air depends on the number and types of people present, thus the quality of the household system and mechanical movement within the enclosed space. In poor houses there are too many people in a small space. Therefore, whatever microbes are shed would lead to a build up of numbers in this confined space.

In the best type of house, there are fewer residents per cubic meter (area) of house space occupied and therefore, shed microbes are spread out in the space available. In addition, the better the house, the better the ventilation to sweeps out microbes from indoor environment.

Cleaning procedures also vary between hospitals and domestic areas and between the different types of domestic environments. The best type of house had many domestic cleaners, good housekeeping procedures and a good air conditioning system.

The similarity in the number of micro-organisms in the hospital and in the very good quality homes is an indicator that both areas have a good hygienic standards.

The source of domestic strains of coagulase negative *Staphylococci* was the environment or from people who had not recently used any anti-microbial agents. Resistance to some antibiotics was detected in some of these strains. This resistance was probably due to the fact that some bacteria are naturally resistant to a particular antibiotic even if they were never exposed to it. However, patients strains are usually subjected or exposed to anti-microbial agents used by hospitalized patients therefore, tend to develop resistance through mutations and adaptation for survival under the environmental conditions which are represented by chemotheraputic agents

The hospital airborne micro-organisms seem to be a mixture of patients', environmental and visitor's strains as their sensitivity falls between domestic and patient strains.

CHAPTER VI

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