An-Najah National University

Faculty of Graduate Studies

Characterization of Indoor Air Bacterial Isolates from Rafidia Hospital, Nablus-Palestine and their Roles in Nosocomial Infections

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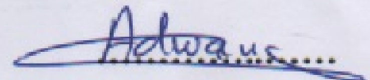
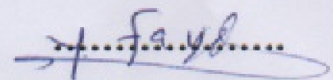
1. Dr. Ghaleb Adwan/ Supervisor	
2. Dr. Yahya Faydi /External examiner	
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Dedication

I wish to dedicate this thesis to my mother; to my late father whose memory continues to inspire me, husband; to my four children Heba, Sondos, Ahmad and Abederahman; to my sisters and brothers. Finally, to my friends and school team for their continuous love and support.

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Lastly, I would like to thank my family and to all my friends of the school for their help and cooperation.

Thank you all ………..

الإقرار

أنا الموقعة أدناه، مقدمة الرسالة التي تحمل العنوان:

Characterization of Indoor Air Bacterial Isolates from Rafidia Hospital, Nablus-Palestine and their Roles in Nosocomial Infections

أقر بأن ما شملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمّت الإشارة إليه حيثما ورد، وأنّ هذه الرسالة ككل، أو أيّ جزء منها لم يقدّم من قبل لنيل أيّ درجة أو لقب علميّ لدى أيّ مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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Abbreviations

ERIC PCR	enterobacterial repetitive intergenic consensus PCR
ICU	intensive care unit
NR	neonatal room
SOR	surgical operation room
CFU	colony forming units
OT	operation theatre
ENT	ear, nose and throat
CoNS	coagulase negative Staphylococci
S. aureus	*Staphylococcus aureus*
P. aeruginosa	*Pseudomonas aeruginosa*
E.coli	*Escherichia coli*
REP	repetitive extragenic palindromic
TSA	tryptic Soy Agar
MSA	mannitol salt agar
MHA	meullar Hinton agar
EMB	eosin Methylene Blue
TSI	triple Sugar Iron
SIM	sulfied Indole Motility
NA	nutrient agar
AHEM	acta Hygienica, Epidemiologica et Microbiologica
CLSI	clinical and Laboratory Standard Institute
NT	not tested
MRSA	methicillin- resistant *S. aureus*
MDR	multi-drug resistant

Characterization of Indoor Air Bacterial Isolates from Rafidia Hospital, Nablus-Palestine and their Roles in Nosocomial Infections

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Abstract

Air can play a vital role as a reservoir for both pathogenic and non pathogenic living microorganisms. Microbial contamination of hospitals air is considered as a source of hospital-associated infections.

The present study aimed to assess microbial profile of air contamination in different wards of Rafidia Hospital, Nablus-Palestine using both active and passive sampling methods, and to ascertain the contribution of some of these microorganisms to infection using cultures from hospitalized patients at the same time using ERIC PCR technique.

Results of this research showed that total viable count of Gram-positive bacteria was the most frequent microorganisms cultured from different wards of the hospital. Coagulase negative Staphylococci (CoNS) and *Micrococcus* spp, were the most predominant among isolates from air samples from all operation rooms, ICU and neonatal room by passive air sampling method. The percentage of CoNS and *Micrococcus* spp in air of surgical operation rooms, intensive care unit and neonatal room ranged from 61.8%-100% and the average was 5158 CFU/m^2/h-20187 CFU/m^2/h. *Staphylococcus aureus* was the most common microorganisms isolated from neonatal room by active air sampling method, the percentage was 35% and the average was 100 CFU/m^3. Total bacterial level range was116

CFU/ m^3-1085 CFU/$m^{3.}$ The percentage of CoNS and *Micrococcus* spp in air of surgical operation rooms, intensive care unit by active air sampling was 58.8%-100% and the average was 70-1080 CFU/ m^3. The results showed that most frequent Colony Forming Units were obtained from Blood agar with a range of 4085 CFU/ m^2/h -8721 CFU/ m^2/h and Tryptic Soy Agar with a range of 2043 CFU/ m^2/h-7935 CFU/ m^2/h by passive air sampling method.

Antimicrobial susceptibility pattern of bacterial isolates revealed that the most effective antibiotics were ciprofloxacin , norfloxacin and tetracycline against *S. aureus*; tetracycline, ciprofloxacin and norfloxacin against CoNS and *Micrococcus* spp and ciprofloxacin, Trimethoprim/Sulfamethoxazole and tetracycline against Bacillus spp.

ERIC PCR profile based on number and size of generated bands revealed that clinical bacterial strains of *S. aureus*, *E. coli* and *Klebsiella* spp were not clonally related to airborne isolates collected at the same time.

Data presented in this study may be valuable to develop interventions to improve microbial indoor air quality in various hospital wardsand also for preventing or decreasing the occurrence of the nosocomial infections.

Chapter One

Introduction

1.1 General Background

Indoor air quality is a term which refers to the air quality within and around buildings and structures especially as it relates to the health and comfort of its occupants. Indoor air can be polluted by various compounds such as carbon monoxide, volatile organic compounds, particulate matter and microbial contaminants (molds, bacteria, viruses) and any action that introduces harmful contaminants into the air within the building. The concern for quality indoor air is necessary especially in institutionalized settings that accommodate a large number of people such as hospitals, nursing homes, prisons, schools, family houses, offices, dispensaries, hotels and classes because contaminated air can cause both mild and severely irritating health conditions (Tambeker *et al*., 2007). The quality of air in hospitals in relation to microbial contamination at a given time period is determined by the quality of air entering into the building, the number of occupants in the building, their physical activities and resultant aerosol generation, human traffic and the efficiency of ventilation (Adebolu and Vhirterhre, 2002).

Nosocomial infections acquired during hospitalization for proper management depend on the characteristics of the microorganisms, with a high risk of being acquired when the healthcare environment is contaminated. Some patients acquire other disease other than the one they were admitted to and this results from contact with a carrier of the pathogen directly or indirectly through inanimate objects. Improper/unhygienic ventilation system can continually be a source of nosocomial infection (Ayliffe *et al.,* 1999). Sneezing has been described as the most vigorous mechanisms of generating millions of airborne microbial infections into the hospital environment (Pasquaria *et al*., 2000). While the larger droplets fall to the ground or nearby surfaces, smaller ones are rapidly evaporated into a non-volatile form where they remain suspended in the air thereby serving as a source of infection when inhaled by other occupants of the hospital including staff. Drug resistant pathogens and fungi strains are of interest in hospitals. Hospitals have the potential for pathogen spread because they have contact with different medical furniture, instruments, air, medical staff and others, although the infections caused by such nosocomial pathogens involves a contaminated environment which should have applied strict safety biosecurity procedures (Mazzali *et al.,* 2003). In addition, it seems that the role of airborne microorganisms in development of hospital-acquired infections has been underestimated because many of these airborne microorganisms cannot be cultured easily (Heidelberg *et al.,* 1997). Moreover, some of the infections resulting from contact rout have resulted from airborne transportation of microorganisms onto surfaces

(Poirot *et al.,* 2007; Bergeron *et al.,* 2007; Abdollahi and **Mahmoudzadeh**, 2012). Although the cause-and-effect relationship between airborne pathogen levels and nosocomial infections is not known yet, it could be hypothesized that lowering the level of these pathogens in the air would result in providing an environment that would help decrease the risk of nosocomial infection (Poirot *et al.,* 2007; Guriz *et al.,* 2008; Wood *et al.,* 2010).

Hospital acquired infection rate varies from 5-10% in the developed countries to 25% or more in developing countries. These infections are mainly caused by microorganisms or surfaces contaminated by the microorganisms or air contaminated with microbial infections nuclei (Odimayo *et al*., 2008). Nosocomial infections can cause severe pneumonia, infections of the urinary tract, bloodstream and other parts of the body. The microorganisms implicated can enter the body through wounds, catheters as well as by inhalation (Prescott *et al*., 1999).

1.2. Air sampling methods

At the moment, the only effective means of quantifying airborne microbes is limited to the count of colony forming units (CFU). The CFU count is the most important parameter, as it measures the live micro-organisms which can multiply. Air samples can be collected in two ways: by active air samplers or by passive air sampling (the settle plates). Both methods are widely used (Pasquarella *et al.,* 2000). In active monitoring a microbiological air sampler physically draws a specific volume of air

through or over a particle collection device which can be a liquid or a solid culture media or a nitrocellulose membrane and the quantity of microorganisms present is measured in CFU/m^3 of air. This system is applicable when the concentration of microorganisms is not very high, such as in an operating theatre and other hospital controlled environments. There are many different types of active samplers on the market, each based on a different design. Unfortunately, there are many drawbacks that make it difficult to interpret correctly the results obtained by these devices. Different active samplers give different results in the same place at the same time (Pasquarella *et al.,* 2000).

Passive monitoring uses “settle plates”, which are standard Petri dishes containing culture media, which are exposed to the air for a given time in order to collect biological particles which “sediment” out and are then incubated. Results are expressed in CFU/plate/time or in CFU/m^2/hour (Napoli *et al.,* 2012). The main criticism of settle plates is that the measured microbial fallout is not at all or is only weakly correlated with the counts determined by other quantitative methods and with a defined volume of the surrounding atmosphere. The settle plate method is still widely used as a simple and inexpensive way to qualitatively assess the environments over prolonged exposure times. Settle plates are not to be used for quantitative estimations of the microbial contamination levels of critical environments. Settle plates are sterile, economical and readily available. The results obtained by settle plates are reproducible and reliable. Many places in an environment can be checked at the same time.

Data collected on settle plates set in different places, by different operators, can be compared and understood (Pasquarella *et al.,* 2000). According to some authors, passive sampling provides a valid risk assessment as it measures the harmful part of the airborne population which falls onto a critical surface, such as in the surgical cut or on the instruments in operating theatres (French *et al.,* 1980).

1.3 Enterobacterial repetitive intergenic consensus (ERIC) PCR

Airborne pathogen levels and their role in nosocomial infections will be evaluated by comparing certain bacterial organisms with cultures from hospitalized patients collected at the same time using Enterobacterial repetitive intergenic consensus PCR (ERIC PCR). ERIC PCR is a PCR-fingerprinting technique but it is not arbitrary because the primer was designed to known target sequence. The primers used in ERIC PCR are complementary to repetitive sequence that highly conserved in bacterial genomic DNA. There are few repetitive sequences have been reported in bacterial genome include enterobacterial repetitive intergenic consensus (ERIC) sequences, repetitive extragenic palindromic (REP) sequences, and BOX elements. The enterobacterial repetitive intergenic consensus (ERIC) sequences are present in many copies in the genomes of different enterobacteria such as *Escherichia coli*, *Salmonella typhimurium* (Hulton *et al*., 1991; Zulkifli *et al*., 2009). These elements are highly conserved at the nucleotide level, 126 bp long, and include a central core inverted repeat. The position of ERIC elements in enterobacterial genomes varies between

different species and has been used as a genetic marker to characterize isolates within a bacterial species (Versalovic *et al*., 1991; Son *et al.,* 2002). In ERIC-PCR a band pattern is obtained by amplification of genomic DNA located between ERIC elements or between ERIC elements and other repetitive DNA sequences. This technique uses consensus primers in the PCR to amplify DNA sequences located between successive repetitive elements such as RRIC sequence for subtyping Gram-negative enteric bacteria (Hulton *et al.*, 1991; Zulkifli *et al*., 2009)

1.4 Literature review

Approximately 10% of the nosocomial infections in both immune-compromised and healthy people are caused by airborne bacteria (Gioffre *et al*., 2007). It has been suggested that many pathogens can survive as bioaerosol, spread considerable distances, and result in infections (Moletta-Denat *et al*., 2010). Thus, recognition of microbial profile and control of microbial contamination of hospital air wards has great importance especially for those infections that an airborne transmission is postulated (Perdelli *et al*., 2006). The quality of indoor air depends on external and internal sources, such as ventilation, cleaning procedures, the surgical team and their activity (Fleischer *et al*., 2005).

In one study, air samples from ten conventionally ventilated operating rooms were taken simultaneously by the sedimentation method and by the air sampler. The most commonly isolated pathogenic species of bacteria were: *Staphylococcus aureus* (*S. aureus*), *Enterococcus spp*., *Streptococcus*

spp., *Pseudomonas aeruginosa* (*P. aeruginosa*), *Acinetobacter lwoffii* and *Alcaligenes faecalis*. The dominant fungal species were *Penicillium* spp. and *Cladosporium* spp (Fleischer *et al*., 2005). Air samples from seven different operation theatres were processed and the isolates were S. *aureus*, Coagulase negative Staphylococci (CoNS), *Acinetobacter* spp. and *Klebsiella* spp. The dominant bacterial species identified were CoNS (Kaur and Hans, 2007). Javed *et al.,* (2008) showed that *S. aureus* was isolated from all the air samples obtained from the various operation theatres (OTs) except ENT (ear, nose and throat). Coagulase negative staphylococci were isolated from air samples from all the OT with the lowest prevalence in eye (50%) and urology (48%). Other pathogens were also isolated such as *Aspergillus* spp., *Bacillus* spp., and *Streptococcus* spp. In recent study, higher concentration of microorganisms was detected when medical staff was present in the room and investigation or treatment was carried out. The majority of microbial findings in the air were Gram-positive cocci (CoNS, *Micrococcus* spp., *Sarcina* spp.). Findings of Gram-negative stems were sporadic (*Pseudomonas aeruginosa*) as well as the incidence of microscopic fungi (*Cladosporium* spp., *Penicillium* spp.) (Vackova *et al*. 2011). Male medical ward and male surgical general ward showed the highest bacterial and fungal growth while the operating theatre was almost free of microbial burden. The bacteria isolates were *S.aureus*, *Klebsiella* spp., *Bacillus cereus* (*B. cereus*), *B. subtilis*, *Streptococcus pyogenes* and *Serratia marscences* while the fungi isolates included *Aspergillus flavus*, *Penicillium* spp., *Fusarium* spp., *Candida albicans* and

Alternaria spp. *Staphylococcus aureus* was the predominantly isolated bacterium while *Penicillium* sp. was the most isolated fungus (Awosika *et al*., 2012). In other study, the microbial profile of air samples showed that *P. aeruginosa* was the predominantly isolated bacteria from thoracic surgery ward; *S. epidermidis* from bone marrow transplantation ward and neonatal ward; *Enterococcus* from ICU and *Acinetobacter* from operating room. Other microorganisms were also isolated from these wards such as *Proteus*, *Stenotrophomonas maltophilia*, *Enterobacter*, *S. aureus*, *Streptococcus group* D, *E. coli*, *Klebsiella* and *Candida albicans*. *Cladosporium* was the most frequent fungi found (Abdollahi and Mahmoudzadeh, *2012*).

Furthermore, Qudiesat *et al.* (2009) noted that, from their studies in two selected hospitals (a private and a public) in Jordan, the air quality in terms of biological contamination in the governmental hospital was worse than that of the private hospital in all units. In both hospitals, *S. aureus*, *Micrococcus luteus* and CoNS were among the most common bacteria identified whereas fungal species *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp. and *Alternaria* spp. were isolated in both hospitals.

Recently, in cross sectional research from 30 wards in five educational hospitals, the highest fungal populations were *Penicelium* spp. (32.06%), *Cladosporium* spp. (20.5%), *Aspergillus fumigatus* (14.61%) and *A. niger* (7.43%), respectively. The highest bacterial population was coagulase-negative staphylococci (32.49%), *Bacillus* spp. (14.74%), *Micrococcus* spp.

(13.68%) and *Staphylococcus aureus* (11.34%), respectively (Hoseinzadeh *et al*., (2013).

It was reported that the highest bacterial population was recorded in the evening between time 5pm and 6pm compared to the morning and afternoon, ranging from 15cfu/m^3 to 47cfu/m^3 in the Faith Medical Hospital and 17cfu/m^3 to 52cfu/m^3 in the Central Hospital, with the children ward recording the highest bacterial counts of 47cfu/m^3 and 52cfu/m^3 in the Faith Medical Center and Central Hospital, respectively. The concentration of fungal population in air of the five different wards in the two hospitals studied was recorded high in the evening, with values ranging from10 cfu/m^3 to 53 cfu/m^3. Six bacterial species and four fungal genera were identified, among which the bacterial isolates were: *S.aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *Proteus mirabilis* and *Klebsiella aerogenes* and the fungal isolates included *Aspergillus*, *Penicillum*, *Mucor*,and *Fusarium.* The degree of frequency of microbial distribution was high in the bacteriological laboratory and female ward and lowest in the operating room (Ekhaise *et al*., 2008). It was reported from autopsy room air 14 bacterial and 26 fungal species were cultured. Most frequently isolated bacteria were CoNS, *Micrococcus* spp., *Bacillus* spp., and diphtheroid bacillus for the Gram- positive, and *Acinetobacter* spp., *Proteus mirabilis*, and *E. coli* for the Gram-negative groups. Most frequently isolated fungi were *Penicillium* spp., *Alternaria* spp., and *Aspergillus flavus* (Sonmez *et al*., 2011).

1.5 Aims of the study

In Palestinian hospitals there are no previous studies on the prevalence airborne microorganisms. The present study aimed to assess microbial profile of hospital air contamination in different wards of Rafidia Hospital-City of Nablus using both active and passive sampling methods and to further assess the correlation between the results of the different sampling methods. In addition, to ascertain the role of some of these airborne microorganisms in nosocomial infections in cultures collected from hospitalized patients at the same time using ERIC PCR technique.

Chapter Two

Materials and Methods

2.1 Study area and site of samples analysis

Hospital air samples were collected between September and October 2014. The collected samples were cultured, identified and analyzed in Department of Biology and biotechnology, Science College, An-Najah National University, Nablus-Palestine.

2.2 Media preparation

2.2.1Tryptic Soy Agar (TSA)

Tryptic Soy Agar (Acumadia,USA) was prepared according to manufacturer's instructions. In a 1L bottle, 500 ml of deionized water was mixed with 20g TSA, heated and stirred until the agar dissolved. The solution was then autoclaved at 121°C for 15 minutes and allowed to cool to about 45 °C to 50 °C. A 20-25 ml agar was then poured into Petri dishes and the dishes were covered and left overnight at room temperature. After that, Petri dishes were stored at 4°C.

2.2.2 Mannitol salt agar (MSA)

BBL^{TM} Mannitol agar (BD,USA) was prepared according to the manufacturer's instructions. In a 1 L bottle, 500 ml of deionized water was mixed with 55.5g MSA, heated and stirred until the agar dissolved. The solution then autoclaved at 121°C for 15 minutes and allowed to cool to

about 45 °C to 50 °C. A 20-25 ml agar was then poured into Petri dishes and the dishes were covered and left overnight at room temperature. After that, Petri dishes were stored at 4°C.

2.2.3 Meullar Hinton agar (MHA)

Meullar agar (BD,USA) was prepared according to manufacturer's instructions. In a 1 L bottle, 500 ml of deionized water was mixed with 19g MHA and 10g NaCl, heated and stirred until the agar dissolved. The solution then autoclaved at 121°C for 15 minutes and allowed to cool to about 45 °C to 50 °C. A 20-25 ml agar was then poured into Petri dishes and the dishes were covered and left overnight at room temperature. After that, Petri dishes were stored at 4°C.

2.2.4 MacConkey Agar:

MacConkey agar (HIMEDIA,India) was prepared according to the manufacturer's instructions. In a 2 L bottle, 1 L of deionized water was mixed with 49.53g MacConkey, heated and stirred until the agar dissolved. The solution then autoclaved at 121°C for 15 minutes and allowed to cool to about 45 °C to 50 °C.

A 20-25 ml agar was then poured into Petri dishes and the dishes were covered and left overnight at room temperature. After that, Petri dishes were stored at 4°C.

2.2.5 Eosin Methylene Blue (EMB) Agar

EMB medium (HIMEDIA,India) was prepared according to the manufacturer's instructions. In a 2 L bottle, 1 L of deionized water was mixed with 35.96g of EMB agar, heated and stirred until the agar dissolved. The solution then autoclaved at 121°C for 15 minutes and allowed to cool to about 45°C to 50°C. A 20-25 ml agar was then poured into Petri dishes and the dishes were covered and left overnight at room temperature. After that, Petri dishes were stored at 4°C.

2.2.6 Blood agar

A 1L bottle containing deionized water (475mL) and 11.6 g Nutrient Agar were heated and stirred until the agar dissolved. Then, the solution was autoclaved at 121°C for 15 minutes and allowed to cool to about 45°C to 50°C. After that, 25 ml of sterile defibrinated blood was added aseptically and mixed thoroughly. A 20-25 ml agar was then poured into Petri dishes and the dishes were covered and left overnight at room temperature. After that, Petri dishes were stored at 4°C.

2.2.7 Simmons Citrate Agar

Simmons citrate agar (HIMEDIA, India) was prepared according to the manufacturer's instructions. In a 2 L bottle, 1 L of deionized water was mixed with 24.28 g of Simmons citrate, heated and stirred until the agar dissolved. Ten ml of Simmons citrate agar was dispensed into tubes,

autoclaved at 121°C for 15 min. The medium was prepared as slant agar tubes and then stored at 4°C.

2.2.8 Triple Sugar Iron (TSI) Agar

TSI agar (Acumedia, USA) was prepared according to the manufacturer's instructions. In a 2L bottle, 1000ml deionized water was mixed thoroughly with 60 g of Triple sugar iron agar, heated to dissolve the agar. Ten ml of Triple sugar Iron medium was dispensed into tubes, autoclaved at 121°C for 15min. The medium was prepared as slant agar tubes and then stored at 4°C.

2.2.9 Sulfied Indole Motility (SIM) Medium

SIM medium (Acumedia, USA) was prepared according to the manufacturer's instructions. In a 1L bottle, 500ml deionized water was mixed with 15g of SIM agar, heated and stirred until agar dissolved. Medium was dispensed into tubes to a give depth of about 4-5 cm. Then the medium was autoclaved at 121°C for 15min, allowed to cool and then stored at 4°C.

2.3 Identification of microorganisms

2.3.1 Gram staining

Gram staining of bacteria was performed as described previously (Cappiccino and Sherman, 1996).

2.3.2 Mannitol fermentation

Aseptically, a single line of inoculation of test organism was cultured on MSA plate. The plates were then incubated for 24hours at 37ºC (Cappiccino and Sherman, 1996).

2.3.3 Catalase test

Catalase test was carried out by the addition of 40µl of 3% hydrogen peroxide (3 ml of 30% stock hydrogen peroxide concentration with 97 ml sterile water) on bacterial colony cultured on NA or on slide (Cappiccino and Sherman 1996).

2.3.4 Slide coagulase test

One Staphylococcal colony from NA and 100µl of diluted citrated plasma (1 ml citrated plasma with 3 ml sterile normal saline) were mixed on a slide. Agglutination or clumping of cocci within 1minute was considered as positive (Cappiccino and Sherman 1996). Negative samples were further tested by tube coagulase test.

2.3.5 Tube coagulase test

This test was done by inoculating 1ml of diluted (1:4) fresh citrated human plasma with a catalase positive colony from NA in a tube. The tube was then incubated for 18-24 hours at 37ºC and inspected from time to time for the presence of clumping or agglutination (Cappiccino and Sherman 1996).

2.3.6 Citrate utilization test

Citrate utilization test was carried out by inoculation of Simmons citrate agar by means of stab-and streak (Cappiccino and Sherman 1996).

2.3.7 TSI test

TSI test was carried out by inoculation the TSI agar slants by means of stab-and-streak (Cappiccino and Sherman 1996).

2.3.8 Motility test

A well-isolated colony was picked with a sterile needle, and then the SIM medium was stabbed to within 1-2cm of the bottom of the tube. The needle was kept in the same line it entered as it is removed from the medium. The tube was incubated at 35°C for 18-24h (Cappiccino and Sherman 1996).

2.4 Air sampling

2.4.1 Active air sampling

Air was sampled using Air Sampler based on manufacturer instructions. Air was blown on a Tryptic Soy Agar plate as a standard growth medium for bacteria. The flow rate was calibrated at 11.0 L/min and samples of 50 L were collected. Active air sampling was repeated twice in each location and was carried out 2 times weekly for 3 weeks.

Microbial air pollution was evaluated according to the recommendation published in AHEM (Acta Hygienica, Epidemiologica et Microbiologica)

No. 1/2002, State Health Institute, Prague (Vackova *et al*., 2011). The evaluation was carried out in one of the five categories as shown in Table 2.1.

Table 2.1 Categories of microbial indoor air contamination–a concentration criterion of mixed population of bacteria and fungi

Microbial contamination	Bacteria (CFU/ m^3)	Fungi (CFU/ m^3)
very low	< 50	< 25
Low	< 100	< 100
Middle	< 500	< 500
High	< 2000	< 2000
Very High	>2000	>2000

2.4.2 Passive air sampling (sedimentation technique)

Sedimentation technique was carried out as described previously (Awosika *et al*., 2012). This technique was done using open Petri dishes containing different culture media. Duplicate set of plates of each medium (Tryptic Soy Agar, blood agar, MacConkey agar and Mannitol salt agar) were distributed at different sites of wards/units examined. The plates were labeled with sample number, site within the ward and date of sample collection. The plates were placed at 2 chosen sites in the concerned wards at about 1 meter above the ground level. The samplings were done at the morning hours (8.00–12.00 am) two times weekly for 3 weeks. All samples were collected withclosed windows and doors. Samples from operating

room were carried out at different critical sites (near the surgeon, near the surgical instruments) and were left open to the air for one hour during operation times. The plates were covered and transferred immediately to the Microbiology Laboratory- An-Najah N. University for incubation. Plates were incubated at 37 °C for 48 hours; the total numbers of colony forming units (CFU) were enumerated. The identification of the isolates was carried out according to standard procedures.

2.5 Bacterial Identification

Media used by passive and active air sampling were transferred to the laboratory and kept in incubators for 48h at 37°C. The number of CFU/m^{2} was then calculated.. Bacterial colonies were characterized by cultural, morphological and microscopic examination, and further identification was carried out by biochemical tests including: catalase activity, lactose fermentation, mannitol fermentation, blood haemolysis, coagulase test, motility test, TSIand citrate utilization test. Colonies of fungal growth were identified based on colony appearance and microscopic examination.

2.6 Antibiotic susceptibility test

Antimicrobial susceptibility for some bacterial strains was determined according to the Clinical and Laboratory Standard Institute (CLSI) using the disk diffusion method (CLSI, 2011). Some bacterial isolates was examined for resistance using the following antibiotic disks (Oxoid): ciprofloxacin (5 µg), norfloxacin (10µg), Trimethoprim/ Sulfamethoxazole

(1.25/23.75µg), tetracycline (30µg), Cefotaxime (30µg), Oxacillin (1µg), ceftriaxone (30µg), Aztreonam (30µg) and nalidixic acid (30µg). All chemicals or powders /discs were purchased from USA. Zones of inhibition were determined in accordance with procedures of the Clinical and Laboratory Standard Institute (CLSI, 2011).

2.7 DNA extraction and ERIC PCR

2.7.1. DNA Extraction

Total genomic bacterial DNA for certain airborne isolated strains and fromclinical samples collected from inpatients by hospital lab was extracted for PCR as previously described by Adwan *et al*., (2013). Briefly, a loop full of bacterial cells were scraped off an overnight nutrient agar plates, washed twice with 1 ml of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). The pellets were then re-suspended in 500µl of sterile distilled water and boiled for 10-15 min. After that, the suspension was incubated on ice for 5-10 min. Debris were pelleted by centrifugation at 11,500 X *g* for 5 min. DNA concentration was determined using spectrophotometer and the DNA samples stored at -20ºC until further use for ERIC PCR analysis.

2.7.2 ERIC PCR

ERIC (Enterobacterial repetitive intergenic consensus) PCR was performed using Primer ERIC1: 5`-ATG TAA GCT CCT GGG GAT TCA C-3 and Primer ERIC2: 5-AAG TAA GTG ACT GGG GTG AGC G-3. Each PCR

reaction mix (25μL) was carried out using 12.5μL of PCR premix with $MgCl_2$ (ReadyMix™ Taq PCR Reaction Mix with $MgCl_2$, Sigma), 1μM of each primer, 3μL DNA template. In addition, the master mix was modified by increasing the concentration of dNTPs to 400μM, $MgCl_2$ to 3mM and Taq DNA polymerase to 2U. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 2 min at 94ºC will be followed by 30 cycles of initial denaturation 94ºC for 60 s, 40ºC for 120s and 72ºC for 90s, with a final extension step at 72ºC for 5 min. The PCR products were analyzed by gel electrophoresis on 1.7% agarose gel, after that, the gel was stained with ethidium bromide (0.5μg/ml), and then the gel was photographed for further analysis. Fingerprints were compared visually.

2.8 Statistical analysis

Generated data was analyzed by simple mean value and percentages.T-test to differentiate between two sampling methods (A p value of < 0.05 was considered to be dependent)

Chapter Three

Results

Microbial profiles of simultaneous cultures obtained from hospital air samples by active and passive sampling in different wards are presented in Tables 1 and 2. The results indicated that all air samples collected from surgical operation rooms, intensive care unit and neonatal room were contaminated to some extent with different types of microorganisms. The total viable count of Gram-positive bacteria was the most frequent microorganisms cultured from different wards. It was also found that CoNS and *Micrococcus* spp, were the most predominant among isolated bacteria from air samples collected from various wards by passive air sampling method. The percentage of CoNS and *Micrococcus* spp in air of surgical operation rooms, intensive care unit and neonatal room by passive air sampling has ranged from 61.8%-100% and the average was 5158 CFU/ m^2/h-20187 CFU/ m^2/h (Table 1). *Staphylococcus aureus* was the most common microorganisms isolated from neonatal room by active air sampling method, the percentage was 35% and the average was 100 CFU/ m^3. Total bacterial level in these rooms had a range 116CFU/ m^3-1085CFU/$m^{3.}$ The percentage of CoNS and *Micrococcus* spp in air of surgical operation rooms, intensive care unit by active air sampling was 58.8%-100% and the average was 70-1080 CFU/m^3 (Table 2).

The findings of this research showed that most frequent Colony Forming Units were obtained from Blood agar with a range of 4085 CFU/ m^2/h -

8721 CFU/ m^2/h and Tryptic Soy Agar with a range of 2043 CFU/ m^2/h-7935 CFU/ m^2/h by passive air sampling method (Table 3).

The antimicrobial susceptibility pattern of bacteria isolates revealed that the most effective antibiotics were ciprofloxacin , norfloxacin and tetracycline against *S. aureus*; tetracycline, ciprofloxacin and norfloxacin against CoNS and *Micrococcus* spp and ciprofloxacin, Trimethoprim/Sulfamethoxazole and tetracycline against Bacillus spp. The antibiotic resistance profile of the different microorganisms isolated from hospital air in surgical operation rooms, intensive care unit and neonatal room by active and passive air sampling is presented in Table 4.

Results in table 5 indicate the presence of statistically significant difference at (α= 0.05) between the two sampling methods in favor of passive sampling method.

ERIC PCR profile revealed that clinical bacterial strains *S. aureus*, *E. coli* and *Klebsiella* spp. and those isolated from air samples collected at the same time were not clonally related (Figures 1 and 2).

Table 3.1: Microbial air load and spectrum of microbial findings in air samples collected from various hospital wards by passive air sampling.

Room	Average (range) of CFU/m^{2} %						
	S. aureus	**CoNS and *Micrococcus* spp.**	***Bacillus* spp.**	***Corynobacterium* spp.**	***E. coli***	***Klebsiella* spp.**	**Fungi and yeast**
SOR1	668 (0-1335) 6.8%	9152 (5655-12647) 93.2%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
SOR2	0 0.0%	17753 100%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
SOR3	1964 (0-5892) 23.5%	5158 (9898-18381) 61.8%	26 (0-79) 0.3%	53 (0-178) 0.6%	0 0.0%	0 0.0%	1152 (786-1335) 13.8%
SOR4	0 0.0%	11076 86.5%	0 0.0%	0 0.0%	79 0.6%	0 0.0%	1650 12.9%
SOR5	4438 (2121-6756) 17.7%	20187 (17593-22781) 80.4%	118 (79-157) 0.5%	0 0.0%	0 0.0%	0 0.0%	354 (79-628) 1.4%
SOR6	864 (0-1728) 8.6%	8916 (4713-13119) 89.4%	39 (0-79) 0.4%	0 0.0%	0 0.0%	0 0.0%	157 (97-236) 1.6%
ICU	1126 (0-2514) 7.9%	11076 (6520-15004) 78.8%	79 (0-236) 0.6%	681 (0-1728) 4.8%	0 0.0%	26(0-79) 0.2%	1047 (0-2749) 7.3%
NR	1453 (550-2357) 15.4%	6913 (5185-7934) 73.2%	79 (0-157) 0.8%	20 (0-79) 0.2%	20 (0-79) 0.2%	0 0.0%	962 (0-2749) 10.2%

SOR: Surgical operation room; ICU: Intensive care Unit; NR: Neonatal Room; CFU: Colony Forming Unit

Table 3. 2: Microbial air load and spectrum of microbial findings in air samples collected from various hospital wards by active air sampling.

Room	Average (range) of CFU/m^{3} %					
	Total bacterial CFU/ m^{3}	***S. aureus***	**CoNS and/or *Micrococcus* spp.**	***Bacillus* spp.**	***Corynobacteria* spp.**	**Fungi and yeast**
SOR1	570	0 0.0%	570 (50-1090) 100%	0 0.0%	0 0.0%	0 0.0%
SOR2	250	0 0.0%	250 100%	0 0.0%	0 0.0%	0 0.0%
SOR3	116	3 (0-10) 2.3%	103 (20-200) 79.8%	10 (0-20) 7.6%	0	13 (0-40) 10.1%
SOR4	100	30 30%	70 70%	0 0.0%	0 0.0%	0 0.0%
SOR5	1085	0 0.0%	1080 (660-1500) 92.3%	0 0.0%	5 (0-10) 0.43%	85 (30-140) 7.3%
SOR6	100	0 0.0%	100 (0-200) 58.8%	0 0.0%	0 0.0%	70 (0-140) 41.2%
ICU	533	3 (0-10) 0.6%	523 (10-1000) 97.6%	7 (0-10) 1.3%	0 0.0%	3 (0-10) 0.6%
NR	191	100 (0-400) 35%	83 (0-140) 29%	5 (0-20) 1.7%	3 (0-10) 1%	95 (0-380) 33.3%

SOR: Surgical operation room; ICU: Intensive care Unit; NR: Neonatal Room; CFU: Colony Forming Unit;

CNS: Coagulase Nagative Staphylococci

Table 3.3: Colony Forming Unit of air collected from various hospital wards by active and passive air sampling according to the type of medium.

Room	passive air sampling CFU/m^2/h				Active air sampling CFU/m^3
	Blood Agar	Mannitol Salt Agar	Tryptic Soy Agar	Macconkey agar	Tryptic Soy Agar
SOR1	5893	1886	2043	0	570
SOR2	6599	4713	6441	0	250
SOR3	7962	4924	6129	52	108
SOR4	5892	3142	3771	0	100
SOR5	8721	8446	7935	0	947
SOR6	4675	2200	3496	0	160
ICU	4793	3981	4425	52	444
NR	4085	1925	3398	39	200

SOR: Surgical operation room; ICU: Intensive care Unit; NR: Neonatal Room; CFU: Colony Forming Unit.

Table 3. 4: Antibiotic resistance of bacterial isolates recovered from air from various hospital wards by active and passive air sampling

Antibiotics	***S. aureus*** **n =20**	**CoNS and *Micrococcus* spp n =17**	***Bacillus*** **spp. n =10**	***E. coli*** **n =2**	***Klebsiella*** **spp. n =1**	***Corynobacteria*** **spp. n =1**
Ciprofloxacin	35%	17.6%	0%	50%	0%	0%
Norfloxacin	35%	23.5%	NT	NT	NT	NT
Trimethoprim/ Sulfamethoxazole	65%	47%	0%	0%	0%	100%
Tetracycline	10%	5.9%	10%	0%	0%	0%
Cefotaxime	75%	52.9%	60%	0%	0%	100%
Oxacillin	70%	94.1%	NT	NT	NT	NT
Ceftriaxone	NT	NT	60%	100%	0%	0%
Aztreonam	NT	NT	100%	0%	0%	100%
Nalidixic acid	NT	NT	70%	0%	0%	100%

ND: Not tested

Table 3.5: T test to differentiate between active and passive sampling methods.

Sig.	df	t	n	s.d	mean	
*0.000	62	12.85	32	6017.46	14071.9	Passive
			32	399.85	367.9	active

*significant at 0.05 level

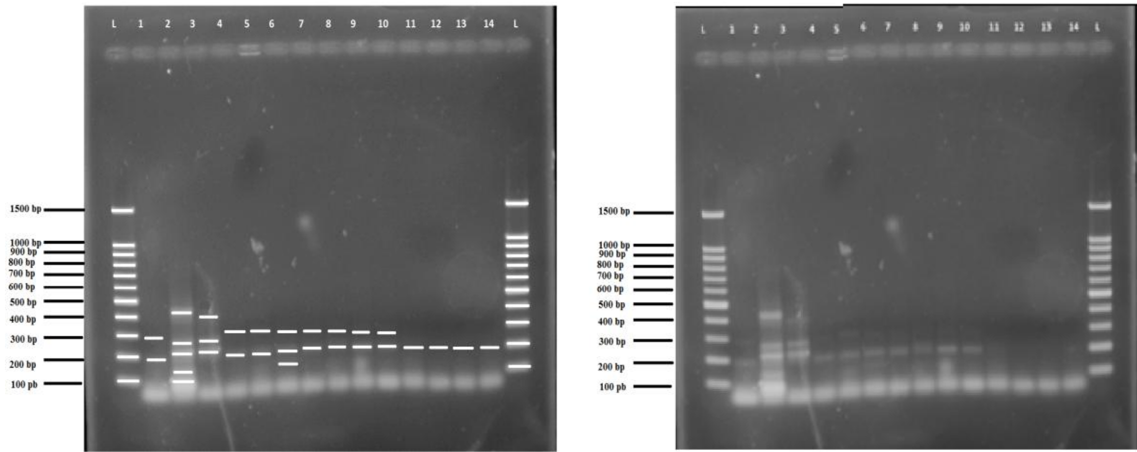

Figure1. DNA fingerprints generated by ERIC PCR analysis of 10 bacterial isolates (*S. aureus* and CNS and/or *Micrococcus* spp.) recovered from clinical and air samples on 1.5% agarose gel. L: 100 bp ladder; lanes 1 and 9 referring to CNS and/or *Micrococcus* spp. isolated from air samples; lanes 2-8 and 10 referring to *S. aureus* isolated from air samples and lanes 10-14 referring to *S. aureus* isolated from clinical samples.

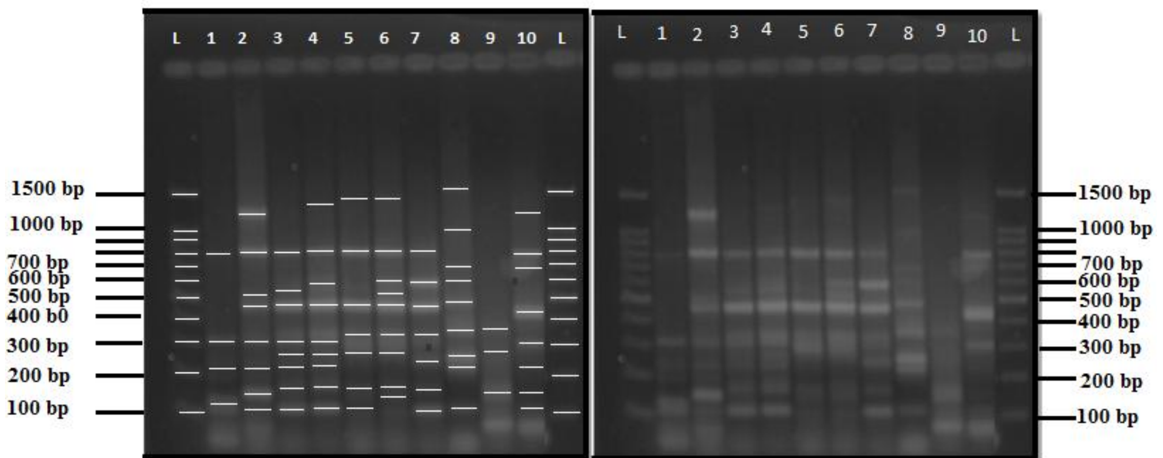

Figure 2. DNA fingerprints generated by ERIC PCR analysis of 10 bacterial isolates (*E. coli* and *Klebsiella* spp.) recovered from clinical and air samples on 1.5% agarose gel. L: 100 bp ladder; lanes 1 and 2 referring to *E. coli* isolated from air; lanes 3-7 referring to *E. coli* isolated from clinical samples; lane 8 referring to *Klebsiella* spp. isolated from air sample and lanes 9 and 10 referring to *Klebsiella* spp. isolated from clinical samples.

Chapter Four

Discussion

Air can play a vital role as a reservoir for both pathogenic and non pathogenic living microorganisms. Microbial contamination of air hospitals is considered as a source of hospital-associated infections (Borghesi *et al*., 2008). These infections may pose high financial load on governments and healthcare systems and may cause considerable morbidity and mortality rate among patients admitted for post-operative surgery, patients in intensive care units with multi-drug resistant strains like methicillin-resistant *S. aureus* (MRSA) show difficult control of these organism (Napoli *et al*., 2012). Microbiological contamination of air in the surgical operation rooms is considered to be one of the most important high risk factor for infections of surgical site in clean surgery (Pasquarella *et al*., 2004). Therefore, recognition, monitoring and control of air microbial contamination of hospital wards is very necessary especially for airborne pathogens postulated to cause hospital-associated infections (Perdelli *et al*., 2006), and can be done routinely by microbiological sampling and particle counting (Javed *et al*., 2008). It could be hypothesized that decreasing the load of these airborne pathogens in the hospital parts could help lower the risk of infection (Guriz *et al*., 2008). There are many factors that play a role to keep the lowest possible airborne microorganism levels in hospital parts. Airborne pathogen levels in hospital parts affected by weather conditions, season, the outdoor microbial load and the number of occupants and visitors, surgical team and their activities, indoor ventilation system

design, cleaning procedures and relative humidity (Sonmez *et al*., 2011; Park *et al*., 2013). Although airborne microorganisms encountered in hospital wards are apparently non pathogenic to healthy people, but they can cause adverse health problems in immune-compromised people. Approximately, it is up to 10% of the hospital acquired infections in both healthy and immunecompromised patients are determined by airborne bacteria (Gioffre *et al*., 2007).

There are 2 most common techniques used for air sampling, the active method carried out by using air sampler and the passive method performed by using settle plate technique. However, there is no accepted opinion about which method is more suitable (Sonmez *et al*., 2011). It was reported that the level of contamination in the air is directly proportional with the number of colonies isolated by passive method (Górny and Dutkiewicz, 2002). The advantages of passive method over active method were reported as cheap, easy to perform, available everywhere, ability to detect and measure harmful part of airborne contamination, many samples can be taken at the same time from different locations, significant outcomes concerning critical surfaces, comparable and usually reliable outcomes, the airflow is not disturbed, and microorganism growth under the natural conditions. The disadvantages of this method can be listed as unknown volume of the sampled air, long sampling time, insufficient for fungal spore evaluation, requirement of large particles, and it is not always accepted by the official guidelines (Pasquarella *et al*., 2000; Sonmez *et al*., 2011). Several studies have been done to compare between the values of microbial

loads obtained by both active and passive methods, in some cases there was no correlation (Petti *et al.*, 2003), while in others there was a significant correlation (Perdelli *et al.*, 2000). Due to air sampling protocols are not consolidated, it is not easy to compare results from different studies (Pasquarella *et al.*, 2008). Both active and passive methods can be used for monitoring and controlling of airborne contamination for a routine surveillance programs in hospitals and medical centers (Napoli *et al.*, 2012). In particular, passive sampling is better to use than active sampling if the air sampling performed to monitor the risk of microbial wound contamination during surgery. On the contrary, the active method should be preferred if the sampling is achieved to assess and evaluate the load of all inhalable viable microorganisms (Napoli *et al.*, 2012).

The load and type of airborne microorganisms in hospitals or their parts can be used to determine the degree of cleanliness. Results from this study showed that indoor hospital air has a low or middle microbial load based on the recommendation published in AHEM (Acta Hygienica, Epidemiologica et Microbiologica) No. 1/2002, State Health Institute, Prague (Vackova *et al.*, 2011). Results showed that 3 of rooms SOR1and SOR5 (during an operation) and ICU had a middle bacterial contamination and total bacterial level ranged 533 CFU/ m^3-1085 CFU/ m^3, while other rooms were of low bacterial contamination and the total bacterial level ranged 116 CFU/ m^3-250 CFU/ m^3. At the same time detection of fungi and yeast in these rooms had a low fungal contamination, based onthe recommendation published in AHEM. The total fungal level ranged from 0 CFU/ $m^3$95 CFU/ m^3. It is

recommended that for conventional operation rooms the microbiological concentration should not be greater than 35 CFU/m^3 in an empty room or 180 CFU/m^3 during an operation. It is also mentioned that for ultra-clean operation rooms the microbiological concentration should be less than 1.0 CFU/m^3 in the middle of an empty room and less than 10 CFU/m^3 during an operation and should not be greater than 20 CFU/m^3 at the periphery. Results showed that the microorganisms load from one ward to another in the same hospital can vary (Abdolahi *et al*., 2009). However, the airborne bacterial load in a modern ventilated operation room should not exceed 30 cfu/ m^3 (Javed *et al*., 2008). A number of studies have been done in operation rooms to determine relation between total airborne bacterial load in operation rooms and risk of infection. It has been observed that loads in the range of 700-1800/m^3 were significantly associated to risk of infection and the risk was slight when the loads were less than 180/m^3 (Parker, 1978). Results of this study could be used to learn the lesson that such microorganisms levels in different hospital wards could be inappropriate; a suitable actions should be taken in order to lower the contamination level and to protect the susceptible people who generally use hospital wards.

Indoor air of hospitals has a wide range of infectious microorganisms (Ekhaise *et al*., 2008; Vackova *et al*., 2011; Sonmez *et al*., 2011; Awosika *et al*., 2012; Hoseinzadeh *et al*., 2013; Gebremariam *et al*., 2015). Results of this report were consistent with previous reports (Kaur and Hans, 2007; Javed *et al*., 2008; Qudiesat *et al.* 2009; Vackova *et al*., 2011; Huseinzadeh *et al*., 2013; Gebremariam *et al*., 2015), which showed that the majority of

bacterial findings in the indoor air were Gram-positive bacteria, Gram-negative bacteria were sporadic as well as incidence of microscopic fungi and yeast. This may be explained that patients, medical staff, seeing of the sick and all the activities in hospital environments as well as surgical operation rooms, intensive care units and neonatal rooms is the main source of the most bacterial organisms (Beegs, 2003; Chow and Yang, 2005; Vackova *et al*., 2011; Park *et al*., 2013). Coagulase-negative staphylococci are opportunistic pathogens which could cause infection in immunocompromised patients. Gram-positive bacteria can survive for long time in the form of aerosol than Gram-negative bacteria. This may be due to differences in cell wall structure. Detection of some kinds of microscopic fungi and yeasts in the indoor air where immunosuppressive patients are treated can be a serious risk factor for the incidence of infectious complication (Soubani *et al*., 2004; Vandewoude *et al*., 2004). In addition, attention to fungal spores presence in hospital air is very important, allergic reactions have been recorded following inhalation of these spores (Omoigberale *et al*., 2014). Humidity and temperature significantly affected fungi loads in air of these rooms (Park *et al*., 2013). *Staphylococcus aureus* is known to be easily harbored in many sites included the throat, nasopharynx, boils, skin, nails and cuts. This microorganism can contribute to the normal microbial flora in the hospital environment causing several infections such as respiratory tract infections, bed sore, post-operative infections and food poisoning under favorable conditions.

Results of this study was in agreement with previous report (Qudiesat *et al.* 2009), which showed that, results from 2 hospitals (a private and a public) in Jordan, *S. aureus*, *Micrococcus* and CoNS were among the most common bacteria identified, whereas fungal species were isolated in both private and a public hospitals. Recently, in cross sectional research from 30 wards in five educational hospitals, it was reported that the highest bacterial population was CoNS, *Bacillus* spp., *Micrococcus* spp. and *S. aureus*, respectively (Hoseinzadeh *et al.,* (2013). *Staphylococcu aureus, S. epidermidis, Micrococus* sp and *Bacillus* sp. were the most frequently occurring airborne bacterial isolates in the two hospitals in Benin City, Nigeria (Ekhaise and Ogboghodo, 2011). Singh *et al.,* 2013 recorded high air contamination in general surgery ward of CoNS 100%, *S. aureus* 66.66% and *Bacillus spp*. 50%, whereas Gynecology ward contain the high contamination of CoNS spp (100%) and *S. aureus* (50%) with least concentration (33.3%) of *Bacillus spp*. These results were consistent with results of this research, which showed that Gram-positive bacteria were the predominant contaminants in these hospital wards. In other study, CoNS, *Micrococcus* spp., *Bacillus* spp., and diphtheroid bacillus were the most frequently isolated microorganisms from autopsy room, respectively, and Gram-positive. *Acinetobacter* spp., *Proteus mirabilis*, and *E. coli* were the most frequently isolated microorganisms.For the Gram-negative groups (Sonmez *et al*., 2011). *Bacillus* species commonly exist in the air, in the soil, in dusty environments and also existing as normal intestinal flora in human and animals. Microorganisms associated to this genus are spore

forming bacteria that can survive for long periods in the environment. *Bacillus* species other than *B. anthracis,* could not cause medical problems except for those suffering from immune deficiency. Diphteroid bacillus as well *Corynebacterium* spp. may exist in the soil, in the air, in the skin and in mucous membranes. However, these species, except *Corynebacterium diphtheriae,* are considered as non pathogenic microorganisms for the patients except for those suffering from immune deficiency (Sonmez *et al*., 2011). In the present study, isolated Gram- negative bacteria; *E. coli and Klebsiella* spp by passive air sampling showed in very low load count. These results were in agreement with recent report (Park *et al*., 2013), which showed very low level of Gram-negative bacteria (17 CFU/ m^3) in hospital lobbies. This may be explained that these bacteria are susceptible for dryness and they are not expected to exist in the air (Sonmez *et al*., 2011). *Klebsiella spp* and *Escherichia coli* are associated with urinary tract infections among catheterized patients.

Antibacteria resistance results in increased illness, death cases and health care costs. Hospitalized patients requiring intensive care and extended treatments are at increased risk hazardous exposure to bacterial air contamination. This risk of health setting infection is increased rapidly by the increasing prevalence of antibiotic-resistant microorganisms and multi-drug resistant (MDR) pathogens such as MRSA (Huang et al., 2013). This study paid particular attention to the presence of drug-resistant species in MRSA and CoNS. In general staphylococcal isolates in this study showed high level of resistance, particularly to Oxacillin. This may indicate that

CoNS could be a natural reservoir for disseminating antibiotic resistance genes including methicillin resistant genes into community (Pamuk *et al*., 2010).

ERIC PCR profile revealed that clinical bacterial strains *S. aureus*, *E. coli* and *Klebsiella* spp and airborne strains collected simultaneously were not clonally related. This might be due to that the short period of the study and not covered all parts of the hospital. A number of studies have indicated that biological indoor air pollutants pose potential hazards to patients, medical staffs, and visitors in hospitals (Javed et al., 2008; Ortiz *et al.*, 2009; Wan *et al.*, 2011; Napoli *et al.*, 2012; Wirtanen et al., 2012; Huang *et al.,* 2013; Omoigberale *et al.,* 2014).

Strategies can be adapted to decrease spreading of microbial contaminants. The quality of indoor air depends on external and internal sources such as cleaning procedures, ventilation, the surgical team and their activities (Zerr *et al*., 2005). This study is considered the first one conducted in Palestine, in order to determine air bacterial isolates in SORs, ICU and NR. More studies are warranted on quality of air in these rooms. These data may be valuable to develop interventions to improve the microbial indoor air quality among different hospital wardsand also for preventing or decreasing the occurrence of the nosocomial infections.

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جامعة النجاح الوطنية

كلية الدراسات العليا

وصف الأنواع البكتيرية المعزولة من هواء الأماكن المغلقة من مستشفى رفيديا، نابلس- فلسطين ودورها في عدوى المستشفيات

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د. غالب عدوان

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في برنامج العلوم الحياتية، بكلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس -فلسطين.

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الملخص

يمكن أن يلعب الهواء دوراً حيوياً كمستودع للكائنات الحية الدقيقة الممرضة وغير الممرضة.وبذلك يعتبر التلوث الميكروبي لهواء المستشفيات مصدر من مصادر العدوى المرتبطة بالمستشفيات.

هدفت الدراسة الحالية لتقييم الملف الميكروبي لتلوث الهواء في الأقسام المختلفة في مستشفى رفيديا – نابلس – فلسطين باستخدام طريقتين لجمع العينات هما الطريقة النشطة (active) والطريقة السلبية (passive) ، والتأكد من مساهمة بعض الكائنات الحية الدقيقة بالعدوى المرتبطة بالمستشفيات عن طريق مقارنتها بعينات مزروعة من مرضى مقيمين في المستشفى في نفس الوقت باستخدام تقنية ERIC PCR.

أظهرت نتائج هذا البحث أن إجمالي عدد البكتيريا الحية الموجبة الجرام المستزرعة من الأقسام المختلفة لمستشفى رفيديا هي الأكثر شيوعا. أيضا أظهرت النتائج أن المكورات العنقودية سلبية التخثر(CoNS) وأنواع الميكروكوكس هي الأكثر سائدة من بين البكتيريا المعزولة من عينات الهواء في غرف العمليات ووحدة العناية المركزة وغرفة حديثي الولادة باستخدام الطريقة السلبية لجمع العينات. وقد تراوحت النسبة المئوية للمكورات العنقودية سلبية التخثر والميكروكوكس في الهواء في غرف العمليات الجراحية ووحدة العناية المركزة وغرفة حديثي الولادة باستخدام الطريقة السلبية لجمع العينات من 61.8 % إلى 100 %، وبلغ المتوسط من 5158 خلية بكتيرية/م²/ساعة إلى 17753 خلية بكتيرية/م²/ساعة. المكورات العنقودية الذهبية كانت الأكثر شيوعاً من بين الكائنات الحية المعزولة من غرفة حديثي الولادة باستخدام الطريقة

النشطة لجمع العينات، وكانت النسبة 100% والمتوسط 100 خلية بكتيرية/م³. بلغ إجمالي المستوى البكتيري في هذه الغرف من 116 خلية بكتيرية/م³ إلى 1085 خلية بكتيرية/م³. وكانت النسبة المئوية للمكورات العنقودية سلبية التخثر وأنواع الميكروكوكس في هواء غرف العمليات الجراحية ووحدة العناية المركزة باستخدام الطريقة النشطة لجمع العينات 58.8 % إلى 100 % وكان المتوسط 100 إلى 1080 خلية بكتيرية/م³. أظهرت نتائج هذا البحث أن اكبر عدد من الخلايا البكتيرية تم الحصول عليه باستخدام Blood Agar بنطاق 4085 خلية بكتيرية/م²/ساعة إلى 8720 خلية بكتيرية/م²/ساعة، ومن Tryptic Soy Agar بنطاق 2013 خلية بكتيرية/م²/ساعة إلى 4085 خلية بكتيرية/م²/ساعة باستخدام الطريقة السلبية لجمع العينات.

أظهر نمط الحساسية لمضادات الميكروبات للبكتيريا المعزولة أن المضادات الحيوية الأكثر فعالية كانت سبيروفلوكساسين ونورفلوكساسين ضد المكورات العنقودية الذهبية، تتراسيكلين وسبيروفلوكساسين ونورفلوكساسين ضد المكورات العنقودية سلبية التخثر وأنواع الميكروكوكس، سبيروفلوكساسين، ميثروبريم/سلفاميثوكسازول وتتراسيكلين ضد البكتيريا العصوية (*Bacillus* spp.).

في هذه الدراسة ، كشف ملف ERIC PCR (بالاعتماد على العدد والوزن الجزيئي للأشرطة) أن السلالات البكتيرية السريرية للمكورات العنقودية الذهبية و *E.coli* وكليبسيلا (*Klebsiella* spp.) وتلك المعزولة من عينات الهواء التي جمعت في نفس الوقت لم تكن من سلالات متطابقة وأنه لايوجد صلة بينها. تعتبر هذه الدراسة الأولى التي أجريت في فلسطين من أجل تحديد بكتيريا الهواء المعزولة من غرف العمليات الجراحية ووحدة العناية المركزة وغرفة حديثي الولادة. هناك ما يبرر المزيد من الدراسات على نوعية الهواء في هذه الغرف. هذه البيانات قد تكون ذات قيمة لتطوير الطرق المتبعة لتحسين جودة الهواء الداخلي من ناحية الميكروبات في غرف العمليات الجراحية ووحدة العناية المركزة وغرفة حديثي الولادة في المستشفى وأيضا لمنع أو تقليل حدوث العدوى المرتبطة بالمستشفيات.