Characterization of Indoor Air Bacterial Isolates from Rafidia Hospital, Nablus-Palestine

Ghaleb Adwan[1*], Eman Abedraboo[2], Kamel Adwan[1] and Suhaila Al-Sheboul[3]

[1]*Department of Biology and Biotechnology, An-Najah National University, P.O.Box (7)-Nablus, Palestine.*
[2]*Department of Environmental Science, Faculty of Graduate Studies, An-Najah National University, P.O.Box (7)-Nablus, Palestine.*
[3]*Department of Medical Laboratory Sciences, Jordan University of Science and Technology, P.O.Box (3030), Irbid, Jordan.*

Authors' contributions

This work was carried out in collaboration between all authors. Authors GA, EA and KA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors GA and EA managed the literature searches and analyses of the study performed. Authors GA, KA and EA managed the experimental process and identified the species of microorganisms. Authors GA and SAS edited and reviewed the manuscript. All authors read and approved the final manuscript.

Article Information

Original Research Article

ABSTRACT

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. This study aimed to assess microbial profile of air contamination in different wards of Rafidia Hospital, City of Nablus-Palestine, during the period September and October 2014, using both an active and a passive sampling methods. Results of this research showed that total viable count of Gram positive bacteria; coagulase-negative staphylococci (CoNS) and *Micrococcus* spp. were the most predominant among isolated bacteria from air samples in all surgical operation rooms (SOR), intensive care unit (ICU) and neonatal room (NR) by passive air sampling method. The percentage of CoNS and *Micrococcus* spp. in air of SORs, ICU and NR by passive air sampling has ranged from 61.8%-100% and the average was 5158 CFU/m^2/h to 17753

_____

*Corresponding author: Email: adwang@najah.edu;

CFU/m^2/h. *Staphylococcus aureus* was the most common microorganisms isolated from the neonatal room by active air sampling method, the percentage was 100% and the average was 100 CFU/m^3. Total bacterial level in these rooms had a range 116 CFU/m^3 to 1085 CFU/m^3. The percentage of CoNS and *Micrococcus* spp. in air from SORs and ICU by active air sampling was 58.8%-100% and the average was 100-1080 CFU/m^3. The finding of this research showed that most frequent CFUs were obtained from Blood agar with a range 4085 CFU/m^2/h to 8721 CFU/m^2/h and Tryptic Soy Agar with a range 2043 CFU/ m^2/h to 7935 CFU/ m^2/h by passive air sampling method. In this study, ERIC PCR profile (number and size of bands) 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. This study is considered the first one to be 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 hospital SORs, ICU and NR and also for preventing or decreasing the occurrence of the nosocomial infections.

1. INTRODUCTION

The awareness for quality indoor air is necessary especially in institutionalized settings that harbor a large number of people such as hospitals, nursing homes, prisons, schools, universities and hotels because contaminated air with harmful pathogens or toxic chemical substances can cause both mild or severe health problems [1]. The quality of indoor air in hospitals is related to microbial contamination at a given time period and depends on external and internal sources, cleaning procedures, number of occupants, their physical activities and resultant aerosol generation, human traffic and the efficiency of ventilation [2-4]. Some patients acquire nosocomial infections during hospitalization and this result through direct or indirect contact with contaminated inanimate objects. Improper/ unhygienic ventilation system in hospitals can continually be a source of hospital acquired infection [5]. Cause-and-effect relationship between airborne pathogen levels and nosocomial infections is not known yet, but it could be hypothesized that decreasing the level of these pathogens in the air would result in providing an environment that would help lower the risk of hospital acquired infection [6,7]. Nosocomial infection rate varies from 5-10% in the developed countries to 25% or more in developing countries. These infections are mainly caused by pathogens or surfaces contaminated by the pathogens or air contaminated with microbial infections nuclei [8].

Recognition of microbial profile and control of microbial contamination of hospital air wards has a considerable importance particularly for airborne infections [9]. In one study, air samples from ten conventionally ventilated surgical operation rooms (SOR) 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 [3]. Air samples from seven different SORs were processed and the bacterial isolates were S*. aureus*, coagulase-negative staphylococci (CoNS), *Acinetobacter* spp. and *Klebsiella* spp. Coagulase-negative staphylococci were the dominant bacterial species isolated from the SORs [10]. Another study, showed that *S. aureus* was isolated from all the air samples obtained from the various SORs except ENT (ear, nose and throat). Coagulase-negative staphylococci were isolated from air samples from all the SORs with the lowest prevalence in eye (50%) and urology (48%). Other pathogens were also isolated such as *Aspergillus* spp., *Bacillus* spp. and *Streptococcus* spp. [11]. 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 bacteria *Pseudomonas aeruginosa* (*P. aeruginosa*) were sporadic as well as the incidence of fungi (*Cladosporium* spp*., Penicillium* spp.) [12]. Male medical ward and male surgical general ward showed the highest

bacterial and fungal growth while the SOR was almost free of microbial load. 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* (*A. flavus*), *Penicillium* spp., *Fusarium* spp., *Candida albicans* (*C. albicans*) and *Alternaria* spp. *Staphylococcus aureus* was the predominantly isolated bacterium while *Penicillium* spp. was the most isolated fungus [13]. In another 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 OSR. Other microorganisms were also isolated from these wards such as *Proteus*, *Stenotrophomonas maltophilia*, *Enterobacter*, *S. aureus*, *Streptococcus group* D, *E. coli*, *Klebsiella* and *C. albicans*. *Cladosporium* was the most frequent fungi found [14]. Furthermore, Qudiesat et al. [15] 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%), *A. 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 [16]. Most frequently isolated bacteria from autopsy room air were CoNS, *Micrococcus* spp., *Bacillus* spp. and diphtheroid bacillus for the Gram-positive, and *Acinetobacter* spp., *Proteus mirabilis* (*P. mirabilis*) and *E. coli* for the Gram-negative groups. Most frequently isolated fungi were *Penicillium* spp., *Alternaria* spp. and *A. flavus* [17].

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 SORs, ICU and neonatal room (NR) 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.

2. 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, city of Nablus-Palestine.

2.2 Air Sampling

2.2.1 Active air sampling

Air was sampled using Air Sampler based on manufacturer instructions (New Askir, Italy). Air was blown on a Tryptic Soy Agar (TSA) 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 contamination was evaluated according to the recommendation published in AHEM (Acta Hygienica, Epidemiologica et Microbiologica) No. 1/2002, State Health Institute, Prague [12].

2.2.2 Passive air sampling (Sedimentation technique)

Sedimentation technique was carried out as described previously [13]. This technique was done using open Petri dishes containing different culture media. Duplicate set of plates of each medium (TSA, blood agar, MacConkey agar and Mannitol salt agar were distributed at different sites of examined SORs, ICU and NR. The plates were placed at 2 chosen sites in the concerned SORs, ICU or NR 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 with closed windows and doors. Samples from SORs were carried out at different critical sites (near the surgeon and the surgical instruments) and were left open to the air for one hour during operation times.

2.3 Bacterial Identification

Media used by passive and active air sampling were transferred immediately to the Microbiology Laboratory-An-Najah National University and incubated at 37℃/48 h. 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 different biochemical tests. Colonies of fungal growth were identified based on colony appearance and microscopic examination using lactophenol-cotton blue dye.

2.4 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 [18]. Some bacterial isolates were examined for resistance using the following antibiotic disks (Oxoid): ciprofloxacin (5 µg), norfloxacin (10 µg), Trimethoprim/ Sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg), Cefotaxime (30 µg), Oxacillin (1 µg), ceftriaxone (30 µg), Aztreonam (30 µg) and nalidixic acid (30 µg). Zones of inhibition were determined in accordance with procedures of the CLSI [18].

2.5 DNA Extraction and Enterobacterial Repetitive Intergenic Consensus-PCR (ERIC-PCR)

Total genomic bacterial DNA for certain airborne isolated strains and from clinical samples collected from inpatients by hospital lab was extracted for PCR according to a method described previously [19]. Briefly, a loop full of bacterial cells was 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 pellet was 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 was pelleted by centrifugation at 11,500 X g for 5 min. DNA concentration was determined using spectrophotometer and the DNA samples stored at -20℃ until further use for ERIC-PCR analysis. ERIC-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` [20]. Each PCR reaction mix (25 µl) composed of 10 mM PCR buffer pH 8.3; 3 mM MgCl2; 0.4 mM of each dNTP; 0.8 µM of each primer; 1.5U of Taq DNA polymerase and 150-200 ng of DNA template. 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 was followed by 40 cycles of denaturation at 94ºC for 60 s, annealing at 50ºC for 40 s and extension at 72℃ for 1 min, with a final extension step at 72℃ 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 (0.5 µg/ml), and then the gel was photographed for further analysis. Fingerprints were compared visually.

2.6 Statistical Analysis

Generated data was analyzed by simple mean value and percentages. T-test to differentiate between two sampling methods (A p value of < 0.01 was considered statistically significant).

3. RESULTS ANS DISCUSSION

3.1 Bioload and Microbial Profile

The bioload and type of airborne microorganisms in hospitals or their parts can be used to determine the degree of cleanliness. In current study, 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 SORs, ICU and NR were contaminated to some extent with different types of microorganisms. Results from this study showed that indoor hospital air has a low to high microbial load based on the recommendation published in AHEM (Acta Hygienica, Epidemiologica et Microbiologica) No. 1/2002, State Health Institute, Prague [12]. Results showed that 3 of the tested rooms of SOR1and SOR5 (during an operation) and ICU had a high microbial contamination and total microbial level ranged 536 CFU/m^3 to 1170 CFU/m^3, SOR2, SOR3, SOR6 and NR had middle microbial contamination and a total microbial level ranged 129 CFU/m^3 to 286 CFU/m^3, while SOR4 had a low microbial contamination and the total bacterial level was 100 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.

Table 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)	9152 (5655-12647)	0	0	0	0	0
	6.8%	93.2%	0.0%	0.0%	0.0%	0.0%	0.0%
SOR2	0	17753	0	0	0	0	0
	0.0%	100%	0.0%	0.0%	0.0%	0.0%	0.0%
SOR3	1964 (0-5892)	5158 (1838-9898)	26 (0-79)	53 (0-178)	0	0	1152 (786-1335)
	23.5%	61.8%	0.3%	0.6%	0.0%	0.0%	13.8%
SOR4	0	11076	0	0	79	0	1650
	0.0%	86.5%	0.0%	0.0%	0.6%	0.0%	12.9%
SOR5	4438 (2121-6756)	20187 (17593-22781)	118 (79-157)	0	0	0	354 (79-628)
	17.7%	80.4%	0.5%	0.0%	0.0%	0.0%	1.4%
SOR6	864 (0-1728)	8916 (4713-13119)	39 (0-79)	0	0	0	157 (97-236)
	8.6%	89.4%	0.4%	0.0%	0.0%	0.0%	1.6%
ICU	1126 (0-2514)	11076 (6520-15004)	79 (0-236)	681 (0-1728)	0	26(0-79)	1047 (0-2749)
	7.9%	78.8%	0.6%	4.8%	0.0%	0.2%	7.3%
NR	1453 (550-2357)	6913 (5185-7934)	79 (0-157)	20 (0-79)	20 (0-79)	0	962 (0-2749)
	15.4%	73.2%	0.8%	0.2%	0.2%	0.0%	10.2%

SOR: Surgical Operation Room;   ICU: Intensive Care Unit; NR: Neonatal Room; CFU: Colony Forming Unit

Table 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 %						Microbial contamination*
	Total bacterial/Fungi CFU/ m^3	*S. aureus*	CoNS and/or *Micrococcus* spp.	*Bacillus* spp.	*Corynobacteria* spp.	Fungi and yeast	
SOR1	570	0	570 (50-1090)	0	0	0	High
		0.0%	100%	0.0%	0.0%	0.0%	
SOR2	250	0	250	0	0	0	Middle
		0.0%	100%	0.0%	0.0%	0.0%	
SOR3	129	3 (0-10)	103 (20-200)	10 (0-20)	0	13 (0-40)	Middle
		2.3%	79.8%	7.6%		10.1%	
SOR4	100	30	70	0	0	0	Low
		30%	70%	0.0%	0.0%	0.0%	
SOR5	1170	0	1080 (660-1500)	0	5 (0-10)	85 (30-140)	High
		0.0%	92.3%	0.0%	0.43%	7.3%	
SOR6	170	0	100 (0-200)	0	0	70 (0-140)	Middle
		0.0%	58.8%	0.0%	0.0%	41.2%	
ICU	536	3 (0-10)	523 (10-1000)	7 (0-10)	0	3 (0-10)	High
		0.6%	97.6%	1.3%	0.0%	0.6%	
NR	286	100 (0-400)	83 (0-140)	5 (0-20)	3 (0-10)	95 (0-380)	Middle
		35%	29%	1.7%	1%	33.3%	

SOR: Surgical Operation Room; ICU: Intensive Care Unit; NR: Neonatal Room; CFU: Colony Forming Unit; CNS: Coagulase-Nagative Staphylococci.
* Categories of microbial indoor air contamination by AHEM [12]

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. However, the airborne bacterial load in a modern ventilated operation room should not exceed 30 CFU/m^3 [11].

In this study, 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 SORs, ICU and NR by passive air sampling has ranged from 61.8%-100% and the average was 5158 $CFU/m^2/h$ to 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 116 CFU/m^3 to 1085 CFU/m^3. The percentage of CoNS and *Micrococcus* spp. in air of SORs, ICU by active air sampling was 58.8%-100% and the average was 70-1080 CFU/m^3 (Table 2). Results indicate that there is statistically significant difference at $P < 0.00001$ between the two sampling methods in favor of passive sampling method. These results were consistent with other studies [12,13], which showed that microbiological load varied between wards in the same hospital. A number of studies have been carried out in SORs to determine relation between total airborne microbial load in these rooms and risk of infection. It has been observed that microbial loads in the range of 700-1800/m^3 were significantly associated with risk of infection and the risk was slight when the microbial loads were less than 180/m^3 [21]. Results of current study could be used to learn the lesson that such microorganisms levels in different hospital wards could be inappropriate; more 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 [12,13, 16,17,22]. Results of this report were consistent with other reports published previously [10,12,15,16,22], 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. Coagulase-negative staphylococci are opportunistic pathogens which could cause infections 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 [23]. In addition, attention to fungal spores presence in hospital air is very important, allergic reactions have been recorded following inhalation of these spores [24]. Humidity and temperature significantly affected fungi loads in air of these rooms [25]. *Staphylococcus aureus* is known to be easily harbored in many sites including 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 a report published recently, showed that the prevalence of *S. aureus* among surgical site infections in the same hospital was 30%, Methicillin resistant *S. aureus* accounted for 33.3% of a total of *S. aureus* isolates. Infection with *S. aureus* is most likely associated with endogenous source as it is a member of the skin and nasal flora and also exogenous source with contamination from environment, surgical instruments or from hands of health workers [26].

Results of this study were in agreement with previous report [15], 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 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 [16]. *Staphylococcus aureus, S. epidermidis, Micrococus* spp. and *Bacillus* spp. were the most frequently occurring airborne bacterial isolates in the two hospitals in Benin City, Nigeria [27]. In recent study, high air contamination was recorded in general surgery ward of CoNS 100%, *S. aureus* 66.7% and *Bacillus* spp. 50%, whereas gynecology ward was contained 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 [28]. In other study, CoNS, *Micrococcus* spp., *Bacillus* spp. and diphtheroid bacillus were the most frequently isolated microorganisms from autopsy room, respectively, for Gram-positive bacteria. *Acinetobacter* spp., *P. mirabilis*, and *E. coli* were the most frequently isolated microorganisms for the Gram-negative bacteria [17]. *Bacillus* spp. commonly exist in the air, in the soil, in dusty environments and also existing as normal intestinal flora in humans as well as animals. Microorganisms associated to this genus are spore forming bacteria that can survive for long periods in the environment. *Bacillus* spp. other than *B. anthracis,* could not cause medical problems except for those suffering from immune deficiency. Diphtheroid 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 [17]. In the present study, isolated Gram-negative bacteria; *E. coli and Klebsiella* spp. by passive air sampling showed 0-79 $CFU/m^2/h$ and 0-26 $CFU/m^2/h$, respectively, while by active air sampling 0 CFU/m^3 for both *E. coli* and *Klebsiella* spp. These results were in agreement with a report published recently [25], 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 [17]. *Klebsiella* spp. and *Escherichia coli* are associated with urinary tract infections among catheterized patients.

The findings of this research showed that most frequent CFUs were obtained from Blood agar with a range of 4085 $CFU/m^2/h$ to 8721 $CFU/m^2/h$ and TSA with a range of 2043 $CFU/m^2/h$ to 7935 $CFU/m^2/h$ by passive air sampling method. This may be due to these are enriched media.

3.2 Antimicrobial Susceptibility Pattern

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 by active and passive air sampling is presented in Table 3. Antibacterial 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 [29]. 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 [30].

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

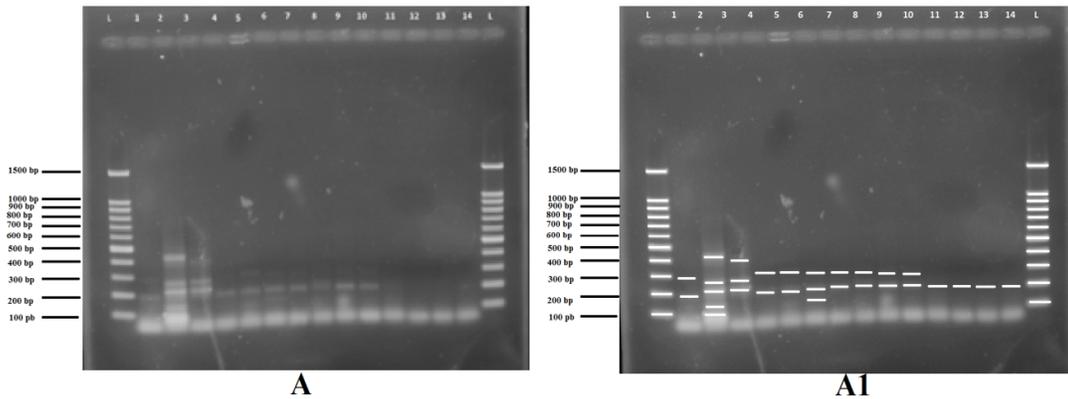| Antibiotics	*S. aureus* n =20	CoNS and *Micrococcus* spp. n =17	*Bacillus* spp. n =10	*E. coli* n =2	*Klebsiella* spp. n =1	*Corynobacteria* spp. n =1
Ciprofloxacin	35%	17.6%	0%	50%	0%	0%
Norfloxacin	35%	23.5%	NT	NT	NT	NT
Trimethoprim/ Sulfamethoxazole	65%	47%	0%	0%	0%	100%
Tetracycline	10%	5.9%	10%	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%

NT: Not tested

Fig. 1. A: DNA fingerprints generated by ERIC-PCR analysis of 14 *S. aureus* isolates recovered from clinical and air samples on 1.5% agarose gel. L: 100 bp ladder; lanes 1, 2, 3 and 6 referring to clinical *S. aureus* isolates; other lanes referring to *S. aureus* isolated from air samples; A1: It is the same as A but bands are demarcated to be obvious
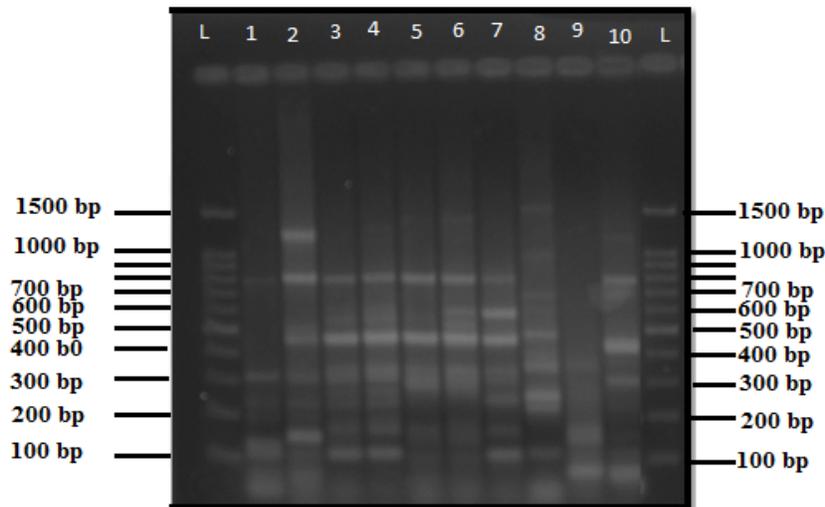

Fig. 2. DNA fingerprints generated by ERIC PCR analysis of 10 bacterial isolates (*E. coli* and *Klebsiella* spp.) recovered from clinical and air samples on 1.5% agarose gel. L: 100 bp ladder; lanes 1 and 2 referring to *E. coli* isolated from air; lanes 3-7 referring to *E. coli* isolated from clinical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

3.3 ERIC-PCR Profile

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 (Figs. 1 and 2 above). This might be due to 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 [11,26,29,31].

4. CONCLUSION

Strategies can be adapted to decrease spreading of microbial contaminants. The quality of indoor air in hospitals is related to microbial contamination at a given time period and depends on external and internal sources, cleaning procedures, number of occupants, their physical activities and resultant aerosol generation, human traffic and the efficiency of ventilation. This study is considered the first one conducted in Palestine, in order to determine air

9

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 wards and also for preventing or decreasing the occurrence of the nosocomial infections.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tambeker DH, Gulhane PB, Bhakare DD. Studies on environmental monitoring of microbial air flora in hospitals. J Med Sci. 2007;7(1):67-72.
2. Adebolu TT, Vhirterhre KJ. Survey of the microbial flora of the Ondo State specialist Hospital Enviroment, Akure, Nigeria. Nig J Microbiol. 2002;16(1120):91-94.
3. Fleischer M, Bober-Gheek B, Bortkiewicz O, Rusiecka-Ziolkowska J. Microbiological control of airborne contamination in hospitals. Indoor Built Environ. 2005;15: 153-156.
4. Zerr DM, Garrision MM, Allpress AL, Heath J, Christakin DA. Infection control policies and hospital-associated infection among surgical patient; variability or association in a multicentre peadiatric setting. Pediatrics. 2005;4:e387-392.
5. Ayliffe GAJ, Babb JR, Taylor LJ. Infection and the spread of microorganisms in hospital acquired infections: Principles and prevention. Butterworth Heinneman Publications, Oxford. 1999;38.
6. Guriz H, Ciftci E, Ayberkin E, Aysev D, Ince E, Arsan S, Yavuz G, Doğru U. *Stenotrophomonas maltophilia* bacteraemia in Turkish children. Ann Trop Paediatr. 2008;28:129-36.
7. Wood GC, Underwood EL, Croce MA, Swanson JM, Fabian TC. Treatment of recurrent *Stenotrophomonas maltophilia* ventilator-associated pneumonia with doxycycline and aerosolized colistin. Ann Pharmacother. 2010;44(10):1665-1668.
8. Odimayo MS, Nwabuisi S, Agegboro B. Hospital acquired infections in Illorin, Nigeria. Trop J Hlt Sci. 2008;15(1):49-54.
9. Perdelli F, Cristina ML, Sartini M, Spagnolo AM, Dallera M, Ottria G, et al. Fungal contamination in hospital environments. Infect Control Hosp Epidemiol. 2006;27:44-47.
10. Kaur N, Hans C. Air bacterial isolations from operation theatres in a tertiary care hospital in India. J Clin Diagn Res. 2007; 1(2):87-89.
11. Javed I, Hafeez R, Zubair M, Anwar MS, Tayyib M, Husnain S. Micobiological surveillance of operation theatres and ICUs of a tertiary care hospital, Lahore. Biomedica. 2008;24:99-102.
12. Vackova M, Hanovcova I, Smetana J, Chlibek R, Bostikova V, Splino M. Microbial air load at the transplant intensive care unit. Mil. Med. Sci. Lett. (Voj. Zdrav. Listy). 2011;80:52-57.
13. Awosika S, Olajubu F, Amusa N. Microbiological assessment of indoor air of a teaching hospital in Nigeria. Asian Pac J Trop Biomed. 2012;2(6):465-468.
14. Abdollahi A, Mahmoudzadeh S. Microbial profile of air contamination in hospital wards. Iran J Pathol. 2012;7(3):177-182.
15. Qudiesat K, Abu-Elteen K, Elkarmi A, Hamad M, Abussaud M. Assessment of airborne pathogens in healthcare settings. Afr J Microbiol Res. 2009;3:66-76.
16. Hoseinzadeh E, Samarghandie MR, Ghiasian SA, Alikhani MY, Roshanaie G. Evaluation of bioaerosols in five educational hospitals wards air in Hamedan, during 2011-2012. Jundishapur J Microbiol. 2013;6(6):e10704. DOI: 10.5812/jjm.10704
17. Sonmez E, Ozdemir HM, Cem EM, Sonmez Y, Salacin S, Ismail OC, et al. Microbiological detection of bacteria and fungi in the autopsy room. Rom J Leg Med. 2011;19(1):33-44.
18. Clinical Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing. Twenty second informational supplement. Wayne, PA, USA: CLSI: M100-S22; 2012.
19. Adwan G, Adwan K, Jarrar N, Salama Y, Barakat A. Prevalence of *seg, seh* and *sei* genes among clinical and nasal *Staphylococcus aureus* isolates. Br Microbiol Res J. 2013;3(2):139-149.
20. Sechi LA, Zanetti S, Dupré I, Delogu G, Fadda G. Enterobacterial repetitive intergenic consensus sequences as molecular targets for typing of *Mycobacterium tuberculosis* strains. J Clin Microbiol. 1998;36(1):128-132.
21. Parker MT. In hospital associated infections, guidelines to laboratory

methods. WHO, Regional Office for Europe, Copenhagen. 1978;28-32.

22. Gebremariam TT, Desta KG, Zelelow YB, Muthupandian S. Microbial load of operating theatre at Ayder Referral Hospital, Northern Ethiopia. Afr J Microbiol Res. 2015;9(9):639-642.

23. Vandewoude KH, Blot SI, Benoit D, Colardyn F, Vogelaers D. Invasive aspergillosis in critically ill patients: Attributable mortality and excesses in length of ICU stay and ventilator dependence. J Hosp Infect. 2004;56(4): 269-276.

24. Omoigberale MNO, Amengialue OO, Iyamu MI. Microbiological assessment of hospital indoor air quality in Ekpoma, Edo State, Nigeria. Global Res J Microbiol. 2014;4(1):1-5.

25. Park DU, Yeom JK, Lee WJ, Lee KM. Assessment of the levels of airborne bacteria, gram-negative bacteria, and fungi in hospital lobbies. Int J Environ Res Public Health. 2013;10(2):541-555.

26. Adwan G, Abu Hasan N, Sabra I, Sabra D, Al-butmah S, Odeh S, et al. Detection of bacterial pathogens in surgical site infections and their antibiotic sensitivity profile. Int J Med Res Health Sci. 2016; 5(5):75-82.

27. Ekhaise FO, Ogboghodo BI. Microbiological indoor and outdoor air quality of two major hospitals in Benin City, Nigeria. Sierra Leone J Biomed Res. 2011; 3(3):169-174.

28. Singh K, Dar FA, Kishor K. Bacterial contamination in operating theatres of District Hospital Budgam in Kashmir division. Innova J Med Health Sci. 2013; 3:62-63.

29. Huang P-Y, Shi Z-Y, Chen C-H, Den W, Huang H-M, Tsai J-J. Airborne and surface-bound microbial contamination in two intensive care units of a medical center in central Taiwan. Aerosol Air Qual Res. 2013;13:1060-1069.

30. Pamuk Ş, Şeker E, Yildirim Y. Antibiotic resistance of coagulase negative Staphylococci isolated from buffalo milk and some milk products. Kocatepe Vet J. 2010;3(2):7-12.

31. Wirtanen G, Nurmi S, Kalliohaka T, Mattila I, Heinonen K, Enbom S, et al. Surface and air cleanliness in operating theatre environments. Europ J Parent Pharmaceut Sci. 2012;17(3):87-93.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16269