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Study of Bacterial Contamination of Indoor Air in Some Wards in National Cancer Institute Sabratha, Libya

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دراسة التلوث الجرثومي في الهواء الداخلي في بعض الأجنحة بالمعهد القومي للسرطان بصبراتة ، ليبيا

الملخص

أجريت هذه الدراسة في المعهد القومي للاورام بصبراتة في ليبيا في الفترة من أغسطس الي اكتوبر 2021 ، باستخدام تقنية الطبق المفتوح لعزل وتوصيف البكتيريا الموجودة في الهواء. تشمل الدراسات الأجنحة التالية: الاستقبال ، جراحة رجال و نساء، العمليات ، الأنسجة ، المناظير، التشخيص ، التصوير الشعاعي ، المختبر ، العيادات الخارجية ، العلاج الكيميائي ، العلاج الإشعاعي ، وأمراض الدم. تراوح نطاق التركيز البكتيري من 416.80 إلى 5500.1 CFU / م ^{3}. تم عزل سلالات بكتيرية معزولة حوالي 108 عزلة بكتيرية وتم تحديدها من جميع الأجنحة الاثني عشر في المستشفى ، وتم تصنيفها على أنها *Staphylococcus aureus 10* (9.25٪) ، المكورات العنقودية الذهبية السلبية 27 .(Coagulase (CoNSA (23.14٪) ، المكورات العنقودية الذهبية المقاومة للميثيسيلين 27 (25.92٪) ، كليبسيلا 7 (6.48٪) ، الإشريكية القولونية 23

(21.29٪) ، العقدية 14 (12.96٪). وقد تسبب هذه البكتيريا بعض الأمراض مثل التهابات الجهاز الهضمي والتهابات الجهاز التنفسي والتهابات المسالك البولية والأمراض الجلدية. تعتبر النتائج المتحصل عليه من هذه الدراسة ذات أهمية قصوى في تنبيه كل من العاملين والمترددين على الأقسام المذكورة في الدراسة إلى خطر انتشار هذه الأنواع البكتيريه وغيرها من مسببات الأمراض.

Abstract

This study was conducted at the National Cancer Institute in Sabratha, Libya, this study was carried out between August 8 and October 10, 2021, using the an open-plate technique to isolate and characterize bacterial air pathogens. The following departments were covered in this study: reception, male and female surgery, operations, histology, endoscopy, diagnostics, radiography, laboratory, outpatient clinics, chemotherapy, radiotherapy, and haematology. The range of the bacterial concentration was 416.80 to 5500.1 CFU/m^3. A total of 108 bacterial isolates were identified from the 12 hospital departments, and classified as: *Staphylococcus aureus* 10(9.25 %), Coagulase negative *Staphylococcus aureus* (CoNSA) 27(23.14%), Methicillin resistance *Staphylococcus aureus* (MRSA) 27(25.92%), Klebsiella spp 7(6.48%), *Escherichia coli* 23(21.29%), *Streptococcus* spp.14(12.96%). These bacteria may cause a variety of illnesses, including gastrointestinal tract infections, respiratory tract infections, urinary tract infections, and skin conditions. These findings would alert the patients, staff and workers to these pathogens and their existence in the wards.

Key word: Bacterial load, *E. coli*, *S. aureus*, MRSA, CoNSA, *Klebsiella* spp, *Streptococcus* spp, Indoor air.

Introduction

Indoor air quality (IAQ) is a term used to describe the quality of air in or around a given building as it pertains to the environment and the health of the people in that area. In fact, it is crucial to focus on having adequate air quality in a healthcare facility where there are plenty of people. Schools, eateries, family homes, banks, hospitals, and other such places are of significant concern

(Tambekeret al., 2007). According to Claudete et al., airborne transmission is a significant method of disease transmission and is to blame for a lot of nosocomial illnesses. (Claudete *et al*., 2006; Rosineide, M. R., & Claudete, F. (2009).

Nosocomial infection, sometimes referred to as the hospital-acquired infection, is an infection picked up in the hospital setting but not when the patient was admitted (Beggs, 2003; Omoigberale et al., 2013). The comprehension of the population of airborne microorganisms in the hospital environment is made possible by the research of airborne bacteria in indoor environments. Hospital-acquired infections, also identified as healthcare-associated infections (HAI), are nosocomially acquired infections that are characteristically not present or might be incubating at the time of admission. These infections are typically acquired after hospitalization and manifest 48 hours after admission to the hospital. The infections are checked carefully by agencies such as the National Healthcare Safety Network (NHSN) of the Center for Disease Control and Prevention (CDC). This investigation is done to prevent HAI and improve patient safety. HAI infections include central line-associated bloodstream infections (CLABSI), surgical site infections (SSI), Hospital-acquired Pneumonia (HAP), catheter-associated urinary tract infections (CAUTI), Clostridium difficile infections (CDI), and Ventilator-associated Pneumonia (VAP) (Ekhaise et al., 2009; 2010; Bove, C. and Kiss, E. 2017; Monegro, et al., 2022). Some of the bacteria have spores, which makes them more resistant and can tolerate a variety of environments (Prigane *et al*., 2004; Odimayo et al., 2008). Additionally, studies have demonstrated that some of the microorganisms in the hospitals inside air can be attributed to the outdoor air. Other indoor sources of airborne germs in a hospital setting include cleaning supplies, products, ventilation systems, and also individual activities (Douwes et al., 2003; Stryjakowska et al., 2007; Luksamijarakul., 2009).

Staphylococcus aureus, *Micrococcus* species, *Pseudomonas* species, *Proteus* species, *Escherichia coli*, *Enterobacter* species, and *Bacillus cereus* are bacteria that are frequently linked to hospital acquired illnesses (Kim et al., 2010; Ekhaise et al., 2008). However,

healthcare personnel are also at danger of contracting an infection in a hospital, in addition to patients and visitors. Moreover, some germs can negatively affect the health of the majority of hospitalized patients, and those with weaker immune systems may be especially vulnerable. (Knibbs et al., 2011). The present study provides information on the indoor air concentrations of bacteria and focuses on the valuation of airborne bacterial communities and identify specific types of bacteria, namely gram positive such as *Staphylococcus aureus* from *Staphylococcus* species and *Streptococcus* species and gram negative like *E. coli* which have high public health significance.

Materials and Methods

Sampling

The open-plate technique was used for this study described by (Bhatia and Vishwakarma, 2010; Ekhaise and Ogboghodo 2010; Agbagwa, O. E and Onyemaechi, S. A. 2014) with some modification. Air Samples were collected from 12 randomly selected units of the hospital namely: Reception, male surgery, female surgery, operations, histology, endoscopy, diagnoses radiology, laboratory, outpatient clinics, chemotherapy, radiotherapy and haematology. The air samples were collected once.

This method lets bacteria-carrying particles settle on the respective culture media. The media sterilized by autoclaving at 121°C for 15 minutes. Prepared plates of MacConkey agar (oxoid) and blood agar (oxoid) were exposed for 1h in the different wards. MacConkey agar (MAC) was used to count total gram-negative bacteria counts, Blood agar was used for the isolation of gram-positive bacteria after sampling, and the dishes were kept in a tightly closed case and taken to microbiology laboratory at National Cancer Institute Sabratha for incubation at 37 °C, for 24-48hours.

Bacterial load

The foreign morphology of the dissimilar colonies formed was noted and identical colonies were sub-cultured into Nutrient Agar (NA), Blood Agar (BA), MacConkey Agar (MAC) plates, incubated suitably, and stored for additional identification and

characterization. (Al-Taweil HI, et al. 2020). Each bacterial colony was identified using standard methods (including colonial morphology, microscopy, and biochemical tests such as: catalase, coagulase, mannitol fermentation and eosin methylene blow test) as described by Cheesbrough M. et al 2006. After incubation of all cultures, at 37C for 48hours, plates were observed in the total number of forming colonies per meter (CFU/m^{-3}). (Soto, T. et al 2009; Cheesbrough M. et al 2006).

As soon as colony forming units (CFU) were counted, colony forming units per meter (CFU /m^3) were determined, taking into account the following equation described by (Fekadu, S. and Getachewu, B. 2015):

$N = 5a \cdot 10^4 (bt)^{-1}$,

Where N: microbial CFU/ m^3 of indoor air;

a: number of colonies per Petri dish;

b: dish surface, cm^2;

t: exposure time, minutes.

Bacterial identification

Identification of bacteria was done macroscopically by colonial morphology, microscopically by Gram stain and Biochemical testes.

Biochemical testes

Catalase test

Catalase enzyme acts as catalyst in hydrogen peroxide to oxygen water. This test is used to differentiate staphylococci from streptococci. 2-3 ml of 3% hydrogen peroxide poured into a test tube. A sterile wooden stick used to remove a good growth of the tested organism and immerse it in the hydrogen peroxide solution. Immediate active bubbling indicated as positive result (Cheesbrough, 2006).

Coagulase test

Coagulase is an enzyme that causes plasma to clot by converting fibrinogen to fibrin when bacteria incubated with plasma. This test used to differentiate coagulase positive *Staphylococcus aureus* from coagulase negative staphylococci. Drop of normal saline on each

end of a slide, a colony of tested organism in each of the drop was mixed to make a thick suspension, a loopful of plasma was added to the suspension and mix gently. Positive result clumping within 10 second (Cheesbrough, 2006).

Mannitol fermentation test

A useful selective medium for *Staphylococcus aureus* which ferments Mannitol produce acid which convert the color of medium from pink to yellow. The tested organism inoculated and incubated at 37˚C overnight. After the period of incubation *Staphylococcus aureus* produce yellow colonies with yellow zones (cheesbrough, 2006).

Eosin methylene blow test

A 100 µl of diluted samples were spread on the surface of the EMBA media (Oxoid CM0069) using sterile bent glass, incubated at 37°C for 24 h. The growing colonies that showed a green metallic colour with black spots in the middle counted as *E. coli* colonies and tested positive (+). Lactose-fermenting gram-negative bacteria acidify the medium, which reduces the pH, and the dye produces a dark purple complex usually associated with a green metallic sheen. This metallic green sheen is an indicator of vigorous lactose and/or sucrose fermentation ability typical of fecal coliforms. Organisms that are slow lactose-fermenters, produce less acid, and the colonies appear brown-pink. Non-lactose fermenters, increase the pH of the medium by deamination of proteins and produce colorless or light pink colonies (CLSI, 2015; Kassim et al., 2016).

Antibiotic sensitivity test

In accordance with recommendations from the Clinical and Laboratory Standards Institute, the disc diffusion method was employed in this investigation (CLSI, 2015; Kassim et al., 2016). All antibiotic disks were bought from Mast Co. Ltd. in the UK and used in accordance with the directions provided by the maker. From the culture plate, two colonies were chosen, emulsified into 2 ml of peptone water broth, and then left for 5 minutes. The excess bacteria suspension was removed before being placed onto the sensitivity

agar. An antibiotic disc containing antibiotic: Oxacillin 1µg, Ciprofloxacin 5µg, Erythromycin 30µg, Nitrofurantoin 300µg, Ticarcillin 75µg, Amoxicillin 30µg, Ampicillin 10µg, Ampillin 30µg, Ceftriaxone 30µg, Fusidic Acid 50µg, Ceftazidime 30µg, Imipenem µg10, Amoxillin 25µg, Cephalothin 30µg, Mezlocillin 75µg, Nalidixic Acid 30µg was placed on each of the culture and incubated for 24hours at 37 °C. The zone of inhibition around each disc was evaluated after the antibiotic had diffused into the medium. Antibiotic-resistant organisms developed around the disc's edge whereas antibiotic-sensitive ones were inhibited at a distance from it. The diameters of zones of inhibition of sensitive organism were measured with meter rule.

Statistical Analyses

The data were analyzed by simple mean value, percentage, and test of significance by using ANOVA to determine significant differences between the bacterial load and different wards by using GraphPad Prisme Version 6.1.

Results and Discussion

A total of 12 wards were sampled for the National Cancer Institute. The total viable count for gram positive and gram-negative bacteria obtained from each area is shown in table 1, The results indicate that the highest bacterial colony forming unit per m^3 air was recorded in female synergic ward was around 5500 CFU/m^3 for gram positive bacteria. While the lowest bacterial colony forming unit per m^3 air was recorded in operation dep. about 416.80 as can be seen on Table 1.

Table 1: Number of gram positive and gram-negative bacterial colony counts (CFU) per m^3 air at morning sampling time at same time of exposure 60 mints.

Sampling sites	Gram positive bacteria (CFU/m^3)	Gram negative bacteria (CFU/m^3)	Total (CFU/m^3)
Reception	**2343.7**	**135.40**	**2479.1**

Male surgery	**1281.2**	**0.0000**	**1281.2**
Female surgery	**5500.1**	**0.0000**	**5500.1**
Operation dep.	**416.80**	**0.0000**	**416.80**
Histology dep.	**1333.3**	**86.450**	**1419.7**
Endoscopy unit	**1781.5**	**0.0000**	**1781.5**
Radiotherapy dep.	**3541.6**	**729.20**	**4270.7**
Diagnosis radiology	**2062.5**	**916.50**	**2979.0**
Outpatient clinics	**635.14**	**0.0000**	**635.14**
Laboratory dep.	**2197.9**	**739.50**	**2937.4**
Haematology	**1260.4**	**604.10**	**1864.5**
Chemotherapy dep.	**2864.5**	**416.60**	**3281.1**

In this study the bacterial concentration of indoor air of National Cancer Institute Sabratha wards was found in the range between 416.80 and 5500.1 CFU/m^3. This range of microbial load is much smaller than that stated from Jima University specialized hospital in which it was estimated among 2123 and 9733 CFU/m^3 (Gizaw, Z., et al 2016). However, the gram-negative bacteria concentration of was from 0.00 to 916.50 CFU/m^3. There is no uniform international standard available on levels and acceptable maximum bioaerosol loads (Jyotshna M, and Helmut B, 2011). Different countries have different standards. The work conducted by a WHO expert group on assessment of health risks of biological agents in indoor environments suggested that total microbial load should not exceed 1000 CFU/m^3 (WHO, 2016). If higher than this, the environment is considered as contaminated (Nevalainen A, and Morawaska L. 2009). Other authors consider that 750 CFU/m^3 should be the limit for bacteria (Francisco RAN, and Luiz FGS 2000; Cappitelli F, et al, 2019). According to these standards the microbial load of National Cancer Institute Sabratha is considered as 'high'. The test showed that there was significant mean bacterial concentration difference among wards (fig1).

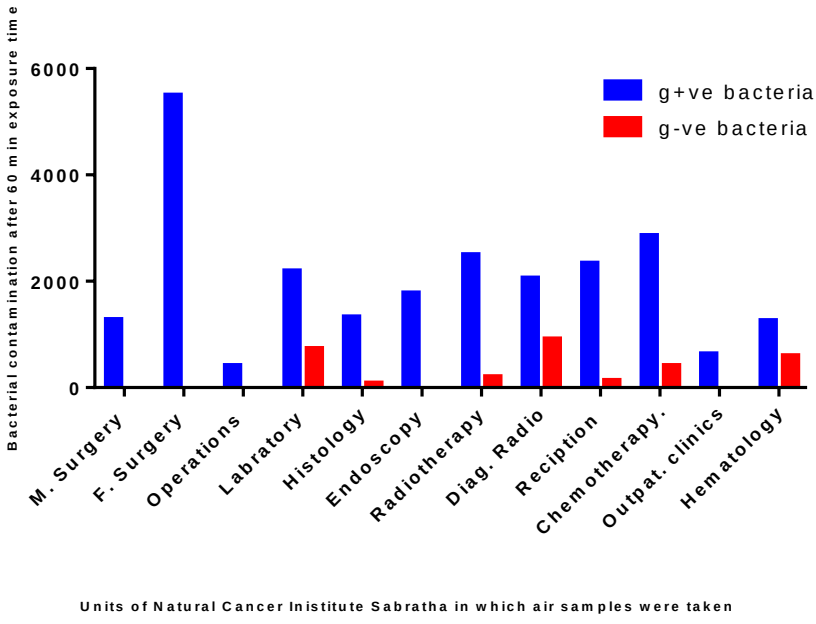

Figure (1): Shown the total viable count for gram positive and gram-negative bacteria obtained from each area.

Number and percentage of isolated bacterial species

The isolates were identified according to morphological appearance, cultural characteristic and biochemical reactions as gram-positive bacteria which further identified as *Staphylococcus aureus* and Coagulase negative *Staphylococcus* (CoNSA), Methicillin resistance *Staphylococcus aureus* (MRSA). The Gram-negative bacteria were identified as *E. coli*, *Klebsiella* sp.

As shown in Table2, 108 organisms were isolated from all the 12 wards of the hospital, these were classified as *Staphylococcus aureus* 10(9.25 %), Coagulase negative *Staphylococcus* (CoNSA) 27(23.14%), Methicillin resistance *Staphylococcus aureus* (MRSA) 27(25.92%), Klebsiella spp 7(6.48%)., *Escherichia coli* 23(21.29%), *Streptococcus* spp.14(12.96%). MRSA and CoNSA were the most frequently isolated bacteria, *Staphylococcus spp.* can

be found everywhere and transmitted from person- to person, from fomites-to-people and also from air-to- people in the hospital. *Staphylococcus* is usually associated in various diseases such as skin infections, urinary tract infections and food poisoning; all these may be responsible for its high occurrence (Huang *et al*., 2006; Zuckerman *et al*., 2009; Ekhaise and Ogbaghdo 2008, 2010; Tang *et al*., 2009).

Table 2: - Number and percentage of bacterial isolated.

Isolates bacteria	Total bacterial count CFU/m^3	Percentage (%)
MRSA	**27**	**25.92%**
Staphylococcus aureus	**10**	**9.25%**
CoNSA	**27**	**23.14%**
***Streptococcus* spp**	**14**	**12.96%**
***Klebsiella* sp**	**7**	**6.48%**
Escherichia coli	**23**	**21.29%**
Total	**108**	

According to Haddadin et al., methicillin resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen that causes severe morbidity and mortality worldwide. Pneumonia and bacteraemia are the majority of MRSA clinical infections. on the other hand, coagulase-negative Staphylococci (CoNS) are among the most frequent constituents of normal skin flora. These organisms are increasingly recognized as agents of clinically significant infection, including bacteremia and endocarditis. Patients at particular risk for CoNSA infection include those with prosthetic devices (eg, pacemakers, intravascular catheters, prosthetic heart valves, orthopedic implants) and immunocompromised hosts. (Tufariello et al., 2020).

Staphylococcus aureus was the one of the dominant isolated organisms, these bacteria is a common pathogenic bacterium that associated with various disease, with multi antibiotics resistance, the presence of this organism might be due to post-sterilization, or the environment contamination. (Tambekar et al. (2007). The isolation of *E. coli* from the wards might be due to faecal contamination and

indicated the possibility of occurrence of different diseases agents especially in immune-compromise persons. According to Beggs (2003), *E. coli*, Klebsiella sp. were the least air contaminants as the source of contamination may be water droplets and not survive for long period.

Antibiotic sensitivity test

Table 3 shows the antimicrobial susceptibility pattern of the bacterial isolates that varied in their sensitivity and resistance patterns of antibiotic used in the study. The isolates showed high sensitivity to Nalidixic acid (75%), Mezlocillin (56%), Ciprofloxacin (26%) and Oxacillin (40%) while Ampillin (82%), Oxacillin (62%), Ceftriaxone (65%), Erythromycin (64%), Amoxycillin (46%), Amoxillin (46%) and Ceftazidime (82%) were highly resistant.

Table 3:Shows the antimicrobial susceptibility pattern of the bacterial isolates that varied in their sensitivity and resistance patterns of antibiotic used in the study.

Antibiotoic	Total No.	Isolated strains Resistance		Isolated Strains Sensitive	
		No.	%	No.	%
Oxacillin(OX1 µg)	108	67	62	43	40
Ampicillin(AMP10µg)	108	18	17	12	11
Imipenem(IMP10 µg)	108	-	-	18	17
Ciprofloxacin(CIP5 µg)	108	-	-	28	26
Piperacillin(PRL30 µg)	108	29	27	14	13
Amoxycillin(AMC30µg)	108	49	46	12	11
Mezlocillin(MEZ75 µg)	108	13	12	61	56
Ticarcillin(TIC75 µg)	108	26	24	05	05
Erythromycin(E30µg)	108	69	64	48	44
Cephalothin(KF30 µg)	108	41	38	05	05

Cefotaxime(CTX30 µg)	108	36	33	08	08
Ampillin (AM30 µg)	108	88	82	14	13
Fusidic acide(FD50 µg)	108	08	08	43	40
Cephalexin(CL30µg	108	27	25	18	17
Ceftriaxone(CRO 30 µg)	108	70	65	37	34
Nalidixic acid(NA30 µg)	108	14	13	81	75
Amoxillin(AML25µg)	108	49	46	12	11
Ceftazidime(CAZ30)	108	88	82	10	09
Colistin(CT10 µg)	108	29	27	20	19
Metronidazole(MET5 µg)	108	28	26	-	-
Azithromycin(AZM15 µg)	108	06	06	18	17
%					

Conclusion

In Conclusion, all wards that were included in the study were heavily contaminated with gram positive bacteria. The high gram-positive bacteria concentrations of air obtained in this study might be potential risk factors for spread of nosocomial infection in the hospital. Thus, immediate interventions are needed to control those environmental factors which favor the growth and multiplication of microbes, and the hospital needs to increase the number of wards to make them sufficient for the inpatients that come from catchment area. It is also vital to control visitors in and out of the wards. Moreover, it is advisable that strict measures be put in place to check the increasing microbial load in the hospital environment.

Additionally, it is necessary to adopt the guidelines for the design and construction of new health-care facilities and for renovation of existing facilities in order to control indoor air-quality. Experiences can be taken from the American Institute of Architects (AIA) that published excellent guidelines. These AIA guidelines address indoor air-quality standards (e.g., ventilation rates, temperature levels, humidity levels, pressure relationships, and minimum air

changes per hour [ACH]) specific to each zone or area in health-care facilities (e.g., operating rooms, laboratories, diagnostic areas, patient-care areas, and support departments) (AIA,2010).

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