

Bacterial contamination of operating room in some of Mosul Hospitals and the effect of ultra-violet light on selected bacteria

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Abstract

This study aimed to isolate and diagnosed the types of bacteria, which is present in the air and surfaces (environments) of the operating theatre in two big hospitals in Mosul (AL-Jamhoury and AL-Kansaa). 629 samples were taken from 9 operating rooms of different speciality through 6 months 258 samples were positive for growth 41.02%, and it showed 401 bacterial growth. Some from isolated bacteria 157 isolation were gram negative by 39.15%, this include 12.97% *Ps. aeruginosa* then followed by *Enterobacteriaceae* 7.98% (which include *E.cloacae* 2.493 %, *E.earogenesa* 1.99%, *E. asburiae* 0.74% *C.freundi* 0.74%, *M.morganii* 0.99%, *S.marcescens* 0.99%) , while *E.coli* was 4.98 % and *Klebsiella* spp 3.74% and 6 isolate of *proteus* spp. by 1.50 %. While Other Gr- bacilli were isolated at 7.98% (*A.baumanii* at 2.406% , *A.faecalis* at 1.74% and un *Pseudomonas* Fluorescent at 4.99%).

Gram positive bacteria were 60.85% isolation, include *staph aureus* was the highest 17.71% and *Staph epidermidis* 6.73% *Staph saprophyticus* 3.74% . There were 43 isolate of *Staphylococcus* (10.72%) including *staph.haemolyticus* 4.738% and *Staph.sihiltera* 2.99%, *Staph.hominis* 1.99 %, *Staph.xyloses* 0.99%. *Bacilli* spp. 9.73% and *Corynebactruim* spp. 1.99%, *Micrococcus* spp. 7.98% while Finally *Streptococcus* spp. were isolated in 2.24% regarding the samples which didn't show any growth it was 58.99% .

UV.C was used for isolated growth. The study shows that UV light can kill the bacteria within 10 min and prevent it for further growth.

Introduction

Contamination of operating theatre is one of the most life threatening sources of nosocomial infection for patients [1]. Nosocomial infection is defined as the infection which is acquired while the patient is in the hospital ,for more than 48 hours it is considered one of the serious complication of hospitalization which increase the morbidity and mortality [2]. There are many factors which lead to (NI), among these the time of exposure ,the way of contamination for the wounds , the degree of sterilization of the equipment's in addition to that the virulence of bacteria and its number and lastly the ventilation system of the operating room. Medical staff and other personnel in addition to patients admitted to theatre can be the source of contamination and

may lead to post-operative wound infection [3]. Increasing personnel number increases the bacteria carrying particles in the operating room [4] Multiple sources of Bacteria have been responsible for hospital contamination (particularly the operating theatre) which include unfiltered air ,ventilation system and antiseptic solution [5]. For these reason ,the (NI) varies from patient to patient in severity and it may lead to death [6] .

Nosocomial infection (NI) became well known problem ,which include 25% of hospital infection in the developing country in addition to increase in the mortality rate [7].

The nosocomial infection is considered very risky when it occur in the intensive care unit (ICU), prematurity or operating rooms[8].

Ultraviolet irradiation has been employed in operating rooms for more than half a century in attempts to reduce airborne bacterial

contamination [9].Ultraviolet light was introduced for operation room for the first time in 1936 in Duke university inDurham in Britain and they found great reduction in bacterialcontamination of the operation room [10], After several decades of negligence of UVGI they start using this method to reduce the air pollution of T.B which is increase during the period (1985_1992) so they start using UVGI for air sterilization [11]. Burge believed that UVGI can cause change or denaturationof the protien of the cells by photochemical effect [10] . The use of UV is one of the preferred method of sterilizing the air of operating room and also sterilizing some of equipments and furniture which cannot be sterilized by the classical method of sterilized like heat or disinfectant solution [12].The use of UV is one of the accepted ecofriend method and it can eliminated the Vegetative bacteria within 15 min and spores within 50 min [13].

Methodology

Materials and methods :Six hundred and twenty nine specimen were taken from nine different operating rooms, from Aljamhuri teaching hospital and Alkhansaa teaching hospital in Mosul ,from the period Nov-2012 and May 2013 .The specimen include :

1-Air samples taken from the operating rooms by putting plate of blood agar opened for half an hour at about one meter height from the floor and then transferred immediately to the hospital Lab. And incubated aerobically at37C° for 24-48 hours .

2 -other envirmetal surfaces : (Floor, walls, tables , operating trolley ,sink, clothes ,anesthesia trolley ,mask ,bed ,lining ,surgical equipment , antiseptic solution and ventilation ducts)

Here we took smears which embedded in sterile normal saline and then transferred to hospital lab for culture on blood agar and

MacConky and incubated aerobically for 24 to 48 hours at 37 C° . The specimen were collected twice weekly at the beginning of the week(on Saturday) and at the end of the week on Wednesday orThursday from each operating room .

For the diagnosis of bacteria :

We depend on the culture characters according to special features of the colony regarding the size and height of the edges and its color .In addition to that we prepare thin smear and stained it with gran stain and then we examine the shape of the cell and the liability for the gram stain [14] .

Biochemical tests :Several test were used to differentiate between these isolated Bacteria such as Coagulase test , Cytochrome oxidase ,growth on manitol salt agar , DNASE , sugar fermentation ,urase test ,IMViC test ,TSI . We use API for confirmation of the diagnosis of bacteria.

We have choose the most resistant bacteria to antibiotics and antiseptic for both *Pseudomonas aeruginosa* and *staph aureus* and study the effect of U V.C on them .we expose the growth to UV .C with 253.7 nm wave light .

We expose the bacteria growth of the cultivating plate to UV for different period (2,4,6,8,10) min this was compared by controlled sample which is not exposed to UV.C [15].

Results

We have taken 629 Samples from 9 different operating rooms from AL-Jamhoury & AL- khansaa teaching hospital from the period November 2012 up to May 2013 . The specimen were taken from different sites including air ,surfaces and medical equipment . Some of the specimen were taken at the beginning of the week (Saturday)and other specimen were taken at the end of the week (Thursday) as shown in table (1)

The sites of taking specimen were including 14 area including Air ,wall ,floors ,ventilation ducts ,sinks, clothes, beds ,operating trolley , surgical equipment ,suckers , Anesthesia machine , masks , operating table and overlapping sheets.

The types of bacteria isolated :

There were 401 isolations of bacteria according to the characteristic features of the colonies, microscopic exam and the biochemical tests. There were 24 types of bacteria of both Gr + and Gr-- .The diagnosis was compatible with API in confirmation [16,17,18].

Gram negative bacteria were 157 (39.15%) and gram positive were 244(60.85%).

The experiment shows that exposure of the growth for 10 min lead to killing all the bacteria which has exposed to UV.C .

Discussion

In this study we have found significant contamination (growth of bacteria) in all operating theatre which reach 41.01%and this is not surprising if it is compared with a similar study done in Mosul by [19] which showed higher contamination which reached up to 66.221%while [20] found a very low incidence of bacterial contamination in operating room in one of Baghdad hospital which reach a level of 3.7%and 4.0% on two consecutive years2001 and 2002. [21] in Iran found a similar degree of contamination which reach up to 57% while in Nigeria [22] found a higher degree of contamination which reach up to 70% .

This variation in difference centers may be attributed to several factors ,first of all to the number of samples and the way of taking the samples and the selected area of sampling .Another causes ,related to personal hygiene

of the individual person and the community and the standard of living and culture . Also the efficiency of sterilization and cleaning procedures which is used in each particular hospital. [23] believed that the difference depend on the methods used in disinfection and sterilization rather than seasonal variation as somebody believes . [24] recorded increase in the isolation of bacteria in spring season because of increase in temperature and decrease in humidity.

In our study there is 60.85% of gram positive bacteria and 39.15%gram negative bacteria .The result is nearly similar to the result of [25] which is 54.35% gram positive and 45.65% gram negative .also similar result by [26] they found more gram positive than gram negative bacteria .While [19] found more gram negative 51.68% than gram positive 48.32% .

This difference may be relate to the type of whether(seasonal variation) or to the types of resistance of bacteria to the antibiotic and its misuse [27]. Gram negative bacteria has the ability to survive if there is water or biologic matrix or media and its disappearance if there are hot weather. While gram positive bacteria has the ability to resist the dry environment for weeks [28].

Types of isolated bacteria:

Staph aureus was the highest isolation in this study which was 17.70 % it is the highest isolation in all operating room except the E.N.T.look Fig (1) .This result is nearly similar to the study of [26] in India they found isolation of *staph aureus* is the highest isolation in 16%.A similar study done in Mosul teaching hospital by [19] , in which *Staph aureus* isolated at 9.5% out of 32.27%of the gram positive bacteria In her study *Klebsilla* was dominating . [21] in Iran found 13.7%of *staph aureus* and it was not the dominating bactria while [23] had 1%

isolation of *staph aureus* in his study which is mainly from the floor and walls of operating theatres . This variation in the percentage of isolation depend on many factors which include air contamination and types of ventilation used ,the technique of sterilization and disinfection ,the activity of surgical team and staff inside operating room [29,30]. Some may attributed the high incidence of staph aureus to its inhabitant normally in the noses of healthy people who work in operating theatre and can be transfer through hands .These bacteria has the ability to stay for long time and it can survive in dry environment for these reasons it is very common in hospital infection and post-operative wound sepsis and these strains has the ability of resistance to the antibiotics [6,31].

The 2nd most common isolated from the O.R was *Ps. aeruginosa* . It had been isolated in 12.97% ,It was the 2nd dominating bactria after *Staph. aureus* except in the gynecology & emergency rooms Fig (1). [20] reported the same result being the 2nd common isolation in the year 2001 and 2002 in Baghdad teaching hospital with the following percentage 13.4% and 25.0% . A similar result also obtained by [22] In Nigeria where *Ps. aeruginosa* we constitute 13% of the total bacteria in O.R. and ICU. In Niger [32] reported 23.3%. *Ps. aeruginosa* represent the most widely spreading bactria in moist environment especially the air cooler and ventilation ducts . It has the ability to resist the antibiotic and antiseptics (Biocide) and it is considered one of the commonest bacteria that cause hospital infection (NI) and urinary tract infection and burn infection .It has wide enzymatic activities and it can grow on very simple media [6,17]. Both *staph aureus* and *ps. aerogenosa* can survive on dry blood spot for 3-6 months and it can survive for 4 weeks on cutton [33].

Bacillus spp. Which is gram positive and spor forming constituted 9.73% from the isolation Fig (1) . In comparison to [19] who found 16.66% of isolation for *bacillus spp.* Similar to [23] in Japan who found nearly similar percentage 15.3% of this bacteria .while in Iran [21] found 18 % of the isolation while [34] found *bacillus spp.* In 8.2%. *Bacillus spp.* has the ability to resist the dry and inconvenient environment and it survive in the soil water and dusts as it form spores and survive for long time [35].

Corynebacter spp. was isolated at 1.99 % in our study Fig (1) while [23] isolate it in Nagoya Hospital in Japan at 7.2%. He attributed to 3 factors related to its presence in the floor and walls of the theatre ,types of ventilation ,efficiency of methods of sterilization and disinfection and the activities of the staff in the operating theatre ,while [34] had different result which is significantly high around 16.2% . These bacteria is normally inhabitant to soil and air and the current of air turbulence may play role of its presence

Enterobacteriaceae group can be transfer from patient to another in the hospital Fig (1) depending on the general condition of these to get the infection [18]. These bacteria usually cause urinary tract infection [6]. In this study we found the isolation at 7.98%. Our result is consistent with [19] who isolate these bacteria at 6.66% while [32] record this bacteria in private hospital in Nigeria in the operating theatre at 10% .

These *E.Coli* considered a normal flora present in the bowel and colon of patient and present normally in the feecal material and was isolate at 4.98% Fig (1) . It can be spread in the surgical or gynecological ward [20]. These bacteria considered opportunistic bacteria which has the ability to produce toxin. [17,19] considered the present of these bacteria an indication of feecal contamination and poor

hygiene .Some author [36] isolate these bacteria in a very low percentage 0.9% .

Klebsiella spp. was isolated at 3.74 % in our study Fig (1) some researchers isolated these bacteria at a lower level [22] who found *Klebsiella spp.* at 2.2% and [36] isolate it at 1.28% . On the other hand [19] isolate these *spp* at a higher percentage from Mosul operating rooms which reached 15% and it was mainly from sink where the surgical staff wash their hands before and after surgery . [22] believed that even with a low percent isolation *Klebsiella spp.* has a very clinical significance in hospital infection specially in pediatric surgery rooms and particularly in those with low immunity [37] attributed the differences in the percentage of isolation to the differences of the site of sampling and isolation and also to the method used .

Enterobacter spp. were isolated at 7.98% where *E.cloacae* is the most common type 2.49% Fig (1). [21] isolate these bacteria at 3.9% while [22] isolated it at a lower percentage 2.2% .on the other hand [19] record *Enterbacter spp.* at a higher percentage 11.33% which was the 2nd most common isolation after *Klebsiella* ,she attributed this increase to the ability of these bacteria for multiplication at room temperature within 24 hours. This bacteria is considered a normal flora of human bowel and colon .It also present naturally on skin of healthy person it can cause opportunistic infection producing nosocomial infection [17].

Proteus spp. were isolated at a lower percentage in this study 1.5% Fig (1). This is consistent with Ekrame who found at a very low level 0.9% while [19] Isolate these bacteria at 2.33% and [22] at a higher level at 8.6 % .This differences may be related to the method of isolation , or to the number of sampling [21]. Recent study done in Mosul by

[27,38] showed a lower percentage of isolation .

Other members of *Enterobacteriaceae* family like *Serratia* ,*Morganella* and *Citrobacter* were isolated at a very low level . Each one of them didn't exceed 1% of the total isolation Fig (1) .Most of these bacteria were transmitted through the hands of the medical staff and the result is consistent with [19]. [39] found that *Serratia* represent 2% of the hospital infection and it is mainly found in the Intensive Care Unit (ICU) .Hospital infection with Resistant strain of *Serratia spp.* is very difficult to control it in most of the cases [31]. *Morganella morganii* were found in four isolations (0.99%) which is comparable with [19] who found it at nearly similar number of isolation (0.66%) . *C. freundii* found 0.74% in our study while [19] found this bacteria in 2% in her study mainly causing urinary tract infection ,blood septicemia . It is not common cause of hospital infection and sometime related to meningitis in neonate [31].

Isolation of coliform bacteria is an indication of faecal contamination of water supply or lack of personal hygiene [21,40].

Other Gram - *Bacillus spp.* constitute 7.98% and *A.baumannii* represent 2.406% Fig (1).This result is similar to other studies done by [21] in Iran 2.3%, [24] in India 2.03% and [36] in USA (2012) 2.57%.Its presence in Hospital environment has very clinical significance as these bacteria can be transferred from noses and hands of the hospital staff to patients especially those immunocompromised or those in I.C.U or rehabilitation unit [41]. These are very common cause of hospital infection and it is resistant to antibiotics [40].

Other types of Gram - bacilli ,*Alcaligenes* were isolated at 1.74% in this study Fig (1) .This finding is differ from what [19] found . It

was 3.33% among her study . It could be part of a normal flora [40] .It is resistant to some disinfectant and it has been isolated from urine , stool , pus from wounds after surgery [42].

The non flourcent *pseudomonas spp.* like *ps. stutzeri* were isolated at 12.97 % in this study Fig (1) . It is considered a rare type and pathogenic bacteria as it cause septicemia ,respiratory tract infection , urinary tract infection , conjunctivitis and post-operative wound infection [42].

Ps. psedomallei were isolated at a lower percentage 0.99% in our study . But [19] isolate this bacteria at a higher percentage 3%. These bacteria found freely in the moisture in water and soil in tropical country [17] .

Regarding gram positive bacteria which include *Micrococcus spp.* it reaches 7.98% of our isolation Fig (1) while [23] isolated it at 4.5%. while [34] found a different result which 11.3% , This bacteria normally found in the skin and surrounding environment and it can be carried out by air .

Coagulase negative staphylococcus group represent 21.12% of our isolation . This result is nearly similar to the result of [19] which reaches 20.11% ,while other showed a higher percentage of isolation [21] shows 36.1% and [24] 26.7% .These bacteria is normally found in the nose , mucous membrane and skin . It increased with increase motion in the operating theatre by patients ,hospital staff and visitors in theatre . It is shedded from noses or skin [43].It can produce hospital infection when there is decreased immunity or coexisting viral infection .

Staph. epidermidis represent the highest isolation of coagulase negative staph (CoNS) which reaches 6.73% in this study Fig (1) .[19] found it at a lower percentage 2.82% [20] record it at a higher percentage 8.3%and it

represent the 3rd rank in their isolation .They consider it a natural source of contamination as it is present normally in the skin of patient and hospital staff , in addition to heavy contamination of beds , bed linen and surrounding environment in hospital [40].

Staph. haemolyticus represent 4.7% of isolation in this study while [19] had 8% and [43] got 2.2% from hospital environment .

Staph. saprophiticus represent 3.74 %in this study Fig (1) while [19] had a lower percentage of isolation 1.83% .

Streptococcus bacteria were isolated at 2.24% in this study Fig (1) from operating room . This bacteria considered a normal flora of respiratory tract and throat . It has the ability to produce toxin which play role in its pathogenic effects [40] . It is similar to the isolation of [19] but other author like [32] who isolated this bacteria at a higher percentage 18.3%. The infection is usually transmitted through respiratory secretion or droplets or it may be transfer by hands or masks or clothes [17].

These finding varies from hospital to hospital depending on the standard of personal hygiene of the individual and community , the rules used in the disinfection and sterilization and the efficiency of the procedure which is followed in the prevention of contamination by the staff and the efficiency of ventilation system used in that theatre .

Ultra-violet :

The exposure to UV.C shows complete inhibition of the growth when we expose these dishes to UV.C for 10 min while [13] show that 15 minutes is needed to get 99.9% effected killing the vegetables cells . while [15] who use Nanoclave cabinet he noticed drastically reduced of contamination of

vegetable cells and also spores of *Cl. Difficile* from many surfaces which are exposed to UV.C within 6 min look Figure (2) .They considered the Nanoclave cabinet is best tool to sterilize equipment and items which cannot be sterilized by heat or chemical disinfectant so UV.C can be used with good economic advantage look pictures (1,2).

Conclusion

The detection of bacteria varies from hospital to hospital and from operating room to another depending on many factors which include the procedure used in this disinfection and sterilization ,the rules which is followed in the prevention of contamination by the staff , the antibiotics policy by that hospital, the efficiency of ventilation system in the hospital and the design of the O.R. inlet and out let, the number of samples taken from each theatre and the standard of hygiene of the individual and community .Demonstrated use of UV rays for certain period can eliminate bacterial growth, The UV.C can be used to sterilized different item within short period efficiently and with low cost. It can be widely used in operating rooms .

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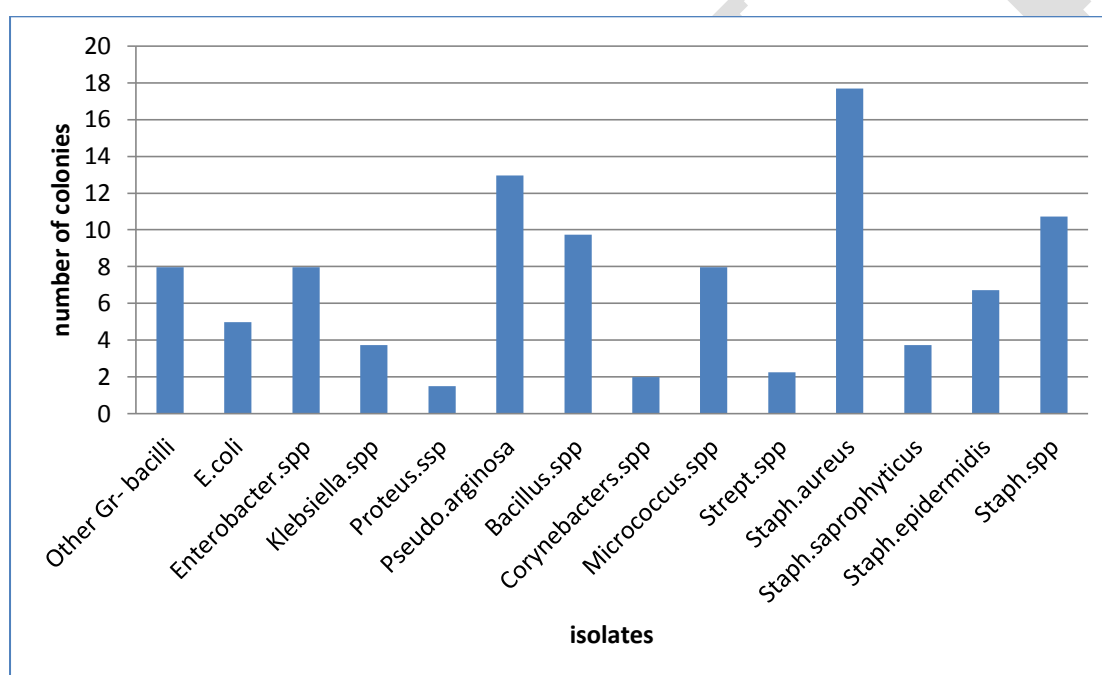
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Table 1. Degree of bacterial contamination at the beginning and end of the week

The end of the week		The beginning of the week		The total number		
Positive bacterial growth(%)	The total samples	Positive bacterial growth(%)	The total samples	Number and ratios of total contamination (%)	The total samples	
(43.8) 28	64	(34.9) 22	63	(39.4) 50	127	General surgery
(56.5) 14	24	(29.6) 8	27	(42.0) 22	51	Urology
(47.6) 10	21	(44.0) 11	25	(45.7) 21	46	Orthopedic
(50) 9	18	(57.9) 11	19	(54.1) 20	37	Emergency
(50) 11	22	(43.5) 10	23	(46.7) 21	45	Burn
(66.7) 14	21	(34.8) 8	23	(50) 22	44	E.N.T
(50.0) 8	16	(23.3) 6	18	(41.2) 14	34	Ophthalmology
(48.4) 31	64	(20.6) 14	68	(34.1) 45	132	Pediatric
(50) 27	54	(27.1) 16	59	(38.1) 43	113	Gynecology
(50) 152	304	(32.62) 106	325	(41.00) 258	629	Total



Fig(1)percentage of types of bacteria isolated

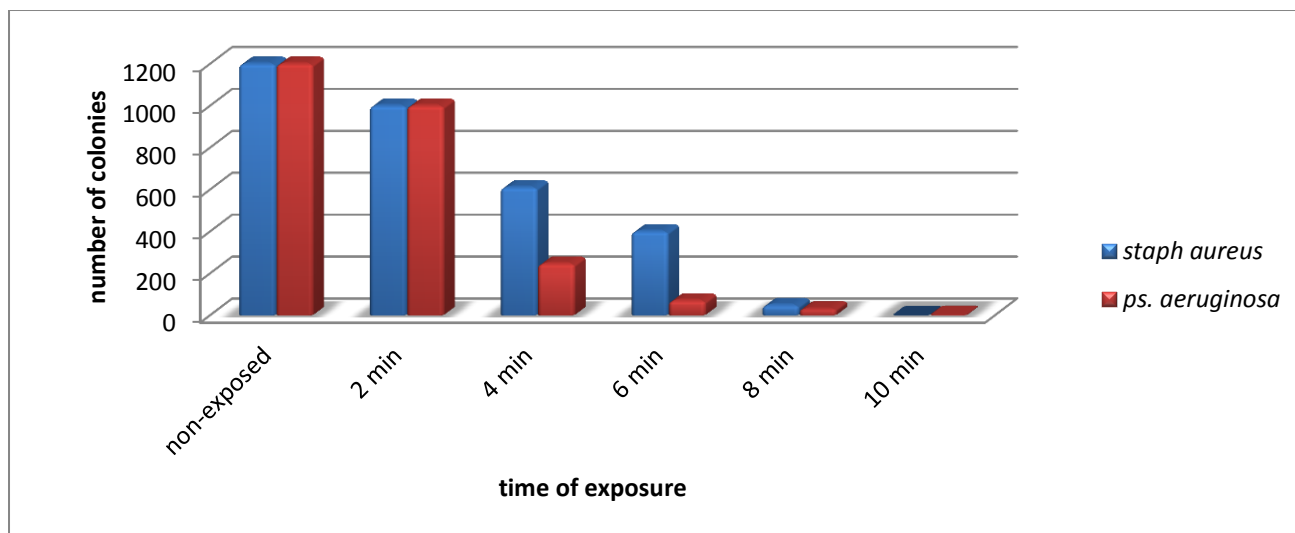
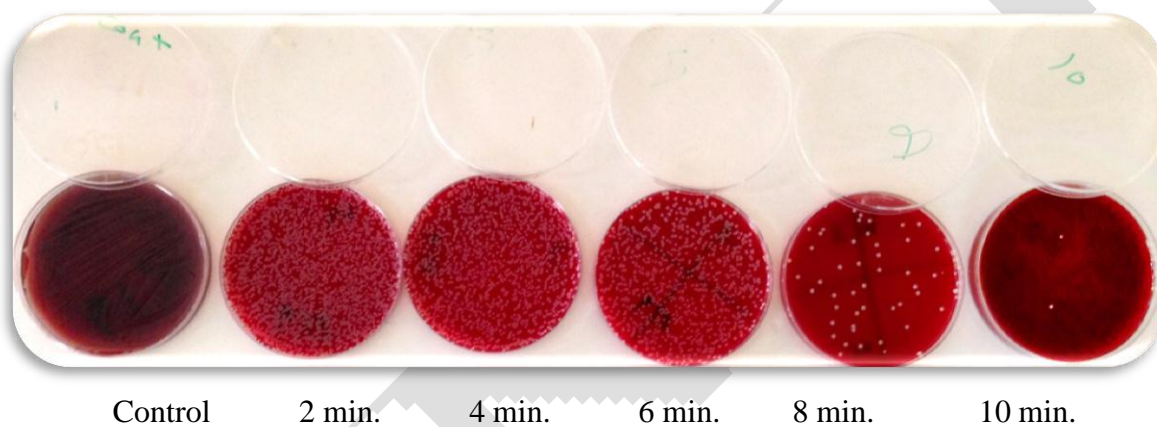
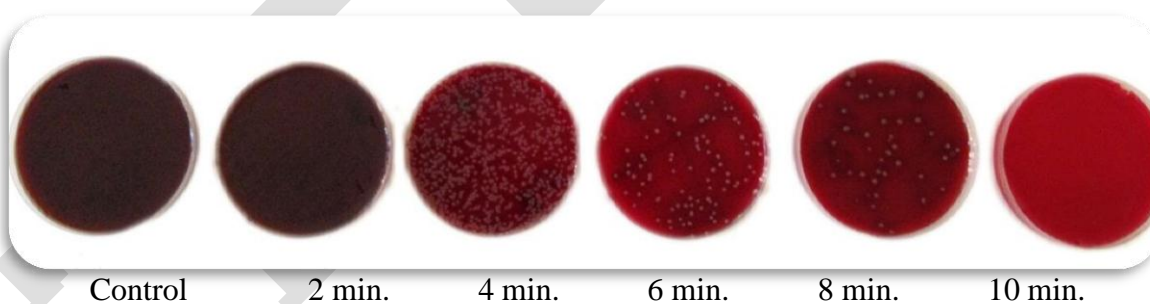


Figure (2): UV light time exposure and killing germs elected



Picture (1) : Exposure time UV light and its impact on *Staph. aureus*



Picture (2) : Exposure time UV light and its impact on *Psedu. aeruginasa*

الخلاصة

الاستاذ المساعد الدكتور ياسين حسين عويد وسمي الجبوري ، جامعة تكريت ، كلية التربية للبنات ، قسم علوم الحياة

زبيدة عبد الرحمن عبد العزيز

هدفت الدراسة الى عزل وتشخيص الجراثيم الموجودة في بيئة صالات العمليات في بداية الاسبوع وقبل اجراء العمليات و بعد اجراء العمليات في مستشفيات في مدينة الموصل (الجمهورية التعليمي،الخنساء التعليمي) اذ تم جمع 629 عينة من الصالات لتسعة اختصاصات وخلال 6 اشهر ،اظهرت 258 عينة نتائج زرع موجبة ونسبة 41.02% ، وتم الحصول على 401 عزلة جرثومية من العينات التي ظهر فيها نمو والتي شخضت باستخدام الفحوصات الشكلية والكيموحيوية اذ كانت منها 157 عزلة سالبة لصبغة كرام ونسبة (39.15%) وتضمنت العزلات السالبة جرثومة الـ *Pseudomonas.aeruginosa* ونسبة 12.97% وبلغت مجموعة البكتريا المعوية الاخرى لـ *Enterobacteriaceae* نسبة 7.98% (اذ تم الحصول على 2.493% من *Enterobacter cloacae* و 1.99% من *Enterobacter aerogenes* و 0.75% من *Enterobacter asburiae* و 0.74% من *Citrobacterfreundii* و 0.99% من *Serratia marcescens* و 0.99% من *Morganellamorganii*)، اما *E.coli* فكانت بنسبة 4.98% و *Klebsiella* بنسبة 3.74% و 6 عزلات للنوع *Proteus spp* بنسبة 1.50% بينما بلغت نسبة عزل العصيات السالبة لصبغة كرام الاخرى 7.98% شملت (*Acinetobacter baumannii* بنسبة 2.24% و *Alcaligenes faecalis* بنسبة 1.74% و 3.99% من جنس *Pseudomonas* غير الفلورنسينية).

اما العينات الموجبة لصبغة كرام فكانت بنسبة 60.85% شملت العصويات الموجبة لصبغة كرام افراد الجنس *Bacillus* اذ تم الحصول عليها بنسبة 9.73% وانواع جنس *Corynebacterium* بنسبة 1.99% كما تم عزل *Micrococcus* بنسبة 7.98% .

اما المكورات العنقودية الذهبية *Staph.aureus* فقد تم الحصول عليها بنسبة 17.70% اما النوع *Staph epidermidis* فقد عزلت بنسبة 6.73% وبلغ نسبة عزل *Staph .saprophyticus* 3.74% بينما بلغت عدد عزلات باقي الانواع للمكورات العنقودية *Staphylococcus* 43 عزلة بنسبة 10.11% (اذ بلغت نسبة *Staph haemolyticus* 4.74% و *Staph schleiferi* بنسبة 2.99% و *Staph.hominis* بنسبة 1.99% و *Staph xylosus* بنسبة 0.99%). اما انواع السبحيات *Streptococcus* فقد عزلت بنسبة 2.24%، اما نسبة العينات التي لم يظهر فيها نمو فكانت 58.99% .

استعملت كابينية الاشعة فوق البنفسجية على بعض النماذج المعزولة واظهرت دراسة تأثير الاشعة فوق البنفسجية على الجراثيم تبين ان زمن 10 دقيقة كان اكثر كفاءة في قتل الجراثيم و منع نموها.