WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 2, 19-34 Research Article ISSN 2278 – 4357

BACTERIOLOGICAL STUDY OF AIR QUALITY IN THE OPERATING THEATERS IN DIYALA GOVERNORATE \ IRAQ

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Article Received on
25 Nov. 2018,
Revised on 16 Dec. 2018,
Accepted on 07 Jan. 2019
DOI: 10.20959/wjpps20192-13057

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ABSTRACT

The study aims at assessing the bacterial load in the air and its relationship with the pollution control program in the operating halls of Baquba Teaching Hospital, Governorate of Diyala / Iraq. a total of 387 samples were collected from December to March 2018, including 270 samples of microbial load of operational halls air (first, second and third surgical hall, main corridor and sub-corridor leading to halls). The samples were collected twice daily as the began of the work in the hall at (8.00 AM 135 samples) and (at midday at 12.00 AM135 samples) by the Active Sampling method, Also 135 swabs were taken within the routine work of the pollution control panels before starting work from (five sites walls, patient beds, anesthesia trolley and fluid retrieval system). The results of microbial culturing of the microbial air load samples showed positive culture in 90% of the plates (270 plates) gave positive results. The colony forming unite ranged from 0-213 CFU and the number of colony-forming units per cubic meter in the air was between 0 - 280 CFU\m^3. The level of microbial load varies according to the time of the air examination and location, as the daily microbial load rated at morning (21.79, 15.44, 30.16, 27.12 and 27.67) for the first, second and third surgical halls and the main and sub-corridor leading respectively and at midday (58.96, 46.07, 72.25, 77.44 and 61.57) respectively. These differences were statistically significant at P level <0.0 5. It gave positive germination dishes to the air samples at the start of work 132 isolation at isolation rate (20.74%) and positive germination dishes at afternoon 168 isolation at isolation rate (62.22%), while the percentage of swabs for pollution control was 20.74% (28/35). The results of bacterial isolation diagnosis from the microbial load of the operations halls showed

that the bacteria Staphylococcus which positive for gram stain accounted for 54% of the isolation aggregate and were distributed to the *Staph auras* 92 isolation (30.66%), followed by *Staph epidermis* 70 isolation (23.33%). While the negative bacillus to gram stain formed a percentage of 46%, *Pseudomonas.ssp* was the most common among the isolation, 46 isolation (15.33%) followed by *Enterobacter.ssp* (42% isolation), *E.coli* 40 isolation (13.33%) and *protuse.ssp* 10 isolation (3.33%). In pollution control swabs, the most common bacteria were *Staph* epidermis 18 isolation (72%) and *Bacillus.ssp* 4 isolation (16%) and *E. coli* 3 isolation (12%). The results of the statistical analysis using ROC curves showed that no correlation between bacterial isolation isolated from air and isolation isolates from control swabs (4.43 and 2.5) respectively.

KEYWORDS: Air sampling, SAS air sampling, Airborne Microorganisms, microbial quality, bio aerosols.

INTRODUCTION

The microorganisms that cause inflammation, as well as the bacteria that make up the spores and the drought resistance, have the ability to spread rapidly in the microbial load of the air, especially in the operating theaters, and this helps to pollute the entire area.[1] More than a century ago, hospital-acquired infections were seen as a critical problem affecting the quality of hospital health care, including surgical, urological, and respiratory infections due to the entry of germs through respiratory tracts.[2] probably 10% of the infections in patients were acquired by them while they are inter to hospital as a result of endemic microorganisms in the hospital environment, resulting in a range of difficulties in postoperative medical treatment.

High rate of microbiology in the internal environment of hospitals causes many acute diseases, infections and allergies.[3] High ratios indicate an indicator of the cleanliness of microbial load in air, Which carries various types of microorganisms.[4] The most common bacteria that commonly causes hospital infections are *Staphylococcus aureus, Escherichia coli*, *Klebsiella*. spp, *Pseudomonas*. spp, *Serratia*. spp, *Proteus*. spp and *Enterobacter*. spp.[5]

Therefore, indoor air quality is of great importance because of the close association between air exposure within hospitals, So hospitals are considered to be the most important internal environments responsible for the spread of airborne diseases.[6]

The study aimed to evaluated microbial load in the operating theaters of Baquba Teaching Hospital, as well as an assessing the models of active air sampling compared with the method of Passive air sampling used in Iraqi hospitals in the control of pollutants in the operating theaters, In addition to isolating and diagnosing aerobic and facultative aerobic bacteria in the air of the operating theaters and comparing them with pollution control swabs.

MATERIALS AND METHODS

Selection of samples

- air samples collecting: 270 samples of air were taken from the operating theaters from different places and different times, including five sites are the first, second and third surgical halls and the main corridor and at the branches leading to the halls, The samples were taken before the start of the official working at 8:00 am and in midday at 11:00 pm during the end of the winter and early spring (14-1 to 1-3) 2018, Using the SAS 100 microbial air sampling device (International pbi Spa Milan / Italy). The device is programmed to pull 500 liters of air.

- Pollution control swabs collecting: 135 swabs were taken from the same places. They included an anesthesia cart, a patient bed, a fluid dispenser, ventilators, walls and floors, sterilizers, disinfectants, surgical instruments.

Samples culture

The samples (air samples, pollution control swabs) were immediately placed on the blood agar medium, maconkey agar and mannitol agar. All dishes were incubated at 37°C for 24 hours, followed by a number of morphological and biochemical diagnostic tests of the identified bacteria.

Diagnosis of bacterial isolates

Macroscopic examination: Bacterial isolates were identified by studying the general vegetative characteristics of the developing colonies on the macconcy, mannitol, and blood agar media, and then the apparent colonies were studied and determined on the basis of texture, color, shape, size, and other general characteristics such as lactose fermentation or not. The presence of blood hemolysis on the blood agar or not.[7]

Microscopic examination: Microscopic examination of bacterial cells was carried out by dyeing them with in gram stain and examined under the oily lens of the optical microscope.

Biochemical tests: Oxidase, Catalase, Indol, Methyl red, Voges-Proskauer, Citrate utilization and Urease tests.

Calculate The Results

The number of colony forming units observed on plates was calculated in cubic meters of hall air from the values of the constituent units of the colonies from 500 liters of hall air, which was prepared according to the statistical equation of the work done by J. Maker [8]. Which was expressed by the statistical probability of passing several particles through the same hole on the surface of the counting dish,the potential number Pr is then used to calculate the number of colony units of CFU per cubic meter of the air sample according to the equation x = (pr x 1000) / v, where pr = cfu per dish, V = 500 liters of air.

Statistical analysis

Data were analyzed using SPSS software (version 20). The ROC test was used to compare the correlations as the level of significance, related to the characteristics of the subjects.

RESULTS AND DISCUTION

Microbial load of the air of the operating halls

The present study is the first in Iraq to be carried which is intended to talke microbial load in the air of the operating halls by active sampling method and its relationship with pollution control swabs on pollution, because the protocol adopted in Iraqi hospitals, depends on the principle of air particles deposition on the surfaces or surfaces of dishes placed in the spaces, Then study the microbes when exist in the Passive sampling method, which gives only qualitative values. The efficiency of the ventilation systems in these clean rooms is not checked as part of the monitoring procedures. Therefore, the standards are established by the World Health Organization.[9]

The results of the present study showed that the total number of units comprising colonies of aerobic and anaerobic bacteria from the air of the operating halls in Baquba Teaching Hospital ranged between 0-228 CFU\m^3 as shown in Table 1.1. This exceeds the limits recommended by the World Health Organization (WHO), which was explained[10], however the number of colony units should not exceed 50 colonies in the morning and 100 colonies at midday. Based on that the percentage of pollution in the operating halls of the hospital in Baquba Teaching Hospital and according to the report of the World Health Assembly is estimated to be "very high". In comparison with a study conducted in Jordan[11], the number

of colony-forming units (0-73 CFU\m^3) (fungus and bacteria), the results of this study were conducted in India by researcher[12] and the number of constituent units of colonies (27-133 CFU \ m^3) (fungus and bacteria).

In comparison with other studies, high levels of air pollution are observed in the halls of Baquba Teaching Hospital, based on this preliminary study, which included only the facultative aerobic and aerobic bacteria, it did not include sports and fungus, which can increase the microbial load recorded if it is included in the study by 30-44%.[13] This work is also important in sterilization techniques used to inhibit or eliminate the growth and breeding of pathogens such as the use of ultraviolet to reduce microbes in the air or change the environment by controlling humidity and temperature, because the possibility of adapting microbes emerging in the air with the new environment in the halls is unlikely. The rate of microbial load varied according to collection time as it was higher at afternoon compared to the morning collection time prior to the start of work for all sites. The average number of colony constituent units in the main corridor air and the secondary corridor at the start of work was (27.57 and 27.12) respectively, (61.57, 77.44), respectively, as shown in Table 1.2.

Table 1.1: Constituent units of colonies per cubic meter at the start of work and at the midday of the surgical halls.

The daily rate of bacterial load	Location and time collection of models									Day of collection
	Third Surgical Hall			Second Surgical Hall			The first surgical theater			
	Daily rate	CFU\m3 Evening	CFU\m3 morning	Daily rate	CFU\m3 Evening	CFU\m3 morning	Daily rate	CFU\m3 Evening	CFU\m3 morning	
22	33	36	30	22	34	10	11	22	0	1
50.33	97	160	34	24	28	20	30	38	22	2
29.33	20	36	4	34	42	26	34	42	26	3
31	47	58	36	10	20	0	36	38	34	4
2	5	6	4	1	2	0	0	0	0	5
15	2	4	0	37	60	14	6	6	6	6
46	44	74	14	33	40	26	61	74	48	7
44.33	40	50	30	80	102	58	13	18	8	8
13.66	7	14	0	7	14	0	27	38	16	9
13	11	22	0	0	0	0	28	40	16	10
16.66	5	10	0	23	30	16	22	30	14	11
20.66	10	14	6	2	4	0	50	64	36	12
11.66	28	46	10	7	12	2	0	0	0	13
77.66	65	84	46	125	200	50	43	44	42	14
64	75	52	98	66	86	46	51	68	34	15
58.66	116	186	46	8	10	6	52	98	6	16
54	62	72	52	27	46	8	74	102	44	17
86	92	174	10	46	68	24	120	206	34	18
25	32	38	26	17	28	6	26	46	6	19
65	96	186	6	63	98	28	36	28	44	20
52.83	25	6	44	60.5	105	16	73	112	34	21
80.33	77	98	56	106	202	10	58	100	16	22
62.5	105	200	10	61	98	24	16.5	29	4	23
76.16	102	108	96	45	56	34	22	33	10	24
68	162	228	96	30	46	14	12	18	6	25
52.5	-	-	-	36	42	30	69	108	30	26

92.5	-	-	-	125	224	26	60	86	34	27
115.5	-	-	-	102	106	98	129	202	56	28
13	-	-	-	-	-	-	13	20	6	29
-	-	**72.25**	**30.16**	-	**46.07**	**15.44**	-	**58.96**	**21.79**	**Mean**

Table 1.2: Constituent units of the colonies per cubic meter at the start of work and at the midday of the two corridors leading to the halls.

Location and time collection of models								
The daily rate of bacterial load	**Sub – corridor**				**The main corridor**			
	The daily average of the sub- corridor	**CFU\m^3 Evening**	**CFU\m^3 morning**	**Day of collection**	**Daily average of the main corridor**	**CFU\m^3 Evening**	**CFU\m^{3}morning**	**Day of collection**
26	28	42	14	1	24	34	14	1
58	86	156	16	2	30	44	16	2
37.5	55	58	52	3	20	36	4	3
33	32	64	0	4	34	52	16	4
40.75	51.5	40	63	5	30	38	22	5
10.5	7	14	0	6	14	28	0	6
3	3	6	0	7	3	6	0	7
93	56	78	34	8	130	202	58	8
36.5	5	10	0	9	68	98	38	9
47	37	60	14	10	57	108	6	10
58	13	16	10	11	103	186	20	11
9	2	4	0	12	16.5	22	10	12
144	111	160	62	13	127	192	62	13
39	23	24	22	14	55	68	42	14
78	79	102	56	15	77	86	68	15
65	59	72	46	16	71	92	50	16
25	35	60	10	17	30	24	6	17
66	26	36	16	18	106	168	44	18

74	78	110	46	19	70	110	30	19
35	38	38	38	20	32	36	28	20
50.5	81	110	52	21	20	4	36	21
52.5	98	108	88	22	7	10	4	22
32	10	10	10	23	54	98	10	23
38.5	15	16	14	24	62	86	38	24
60	38	46	30	25	82	108	56	25
-	23	26	20	26	-	-	-	-
-	0	0	0	27	-	-	-	-
-	111	160	62	28	-	-	-	-
-	-	**61.57**	**27.67**	**Mean**	-	**77.44**	**27.12**	**Mean**
44.62				**Total**	**52.28**			**Total**

Bacterial species isolated from air in the operating halls

The first surgical hall: consisted of 7 isolates of *Staph epidermis* (26.92%), 6 isolations of *Staph auras* (23.07%), 7 isolations of *E. coli* (26.92%) and 2 isolates *Enterobacter.ssp* (7.69%) and 1 isolating *Psedomonas.ssp* (3.84%), isolation of one *Acenetobacter.ssp* (3.84%), and isolation of one *protuse.ssp* (3.84%) as shown in Table 1.3. In the afternoon, the isolates included 30 isolates of different bacteria: 10 of them were *Staph epidermis* (67.85%), 9 of them were *Staph auras* (32.14%), 5 isolated *Psedomonas.ssp* (17.85%) and 7 isolates *Enterobacter.ssp* (23.33%) and 2 *E.coli* was isolated by (7.14%) as shown in Table 1.3.

The second surgical hall: included 27 isolates: 6 isolates of *Staph epidermis* (22.22%), 9 isolates of *Staph auras* (33.33%), 2 isolations of *E. coli* (7.4%) and 6 isolates *Enterobacter.ssp* (22.22%) and isolation One by *Protuse*.ssp by (3.7%) and isolated by Acenetobacter.ssp by (3.7%) and 2 isolates by *Klebsella*.ssp (7.4%) as in Table 1-3. At the peak, developing isolates included 37 isolates (27.4%) for different bacteria, 6 isolates of *Staph epidermis* (16.21%), 12 isolations of *Staph auras* (32.43%), and *Pseudomonas*.ssp isolates (8.1%). *E coli* (16.21%) and 8 isolates *Enterobacter.ssp* by (21.62%) and 2 isolates *Klebsella*.ssp by (5.4%) as in Table 1-3.

Third surgical hall: 27 isolates were isolated when different spores were started: 7 isolates of *Staph epidermis* (25.92%) and 10 isolates of *Staph auras* (37.03%). *Psedomonas.ssp* gave 2 isolates (7.4%), (7.4%), isolation of *E. col* (3.7%), and isolation of one *Klebsella.ssp* (3.7%) as in Table 1.3. At peak, isolates included isolates (14.81%), isolates of *Acentobacter. ssp* (18.42%), *Staph epidermides,* 16 isolates (42.1%), *Staph auras*, 7 isolates (18.42%), *Psedomonas.ssp*, 5 isolates (13.15%), *Enterobacter.ssp* 2 isolates by (5.26%) *E.coli* and one isolate by (2.63%) *Klebsella.ssp* as in Table 1-3.

The main Corridor: Developing isolates included 25 isolates at the onset of different bacterial strains: 5 isolates of *Staph epidermis* (20%), 10 isolates of *Staph auras* (40%), 4 isolates *Enterobacter.ssp* (16%), 5 isolates of *Psedomonas.ssp* (20%) and isolation of *one E. coli* (4%) as in Table 1-3. At the peak, the results were 25 isolates of different bacteria: 2 isolates of *Staph epidermis* (8%), 5 isolates of *Staph auras* (20%), 3 isolates *Enterobacter.ssp* (12%), 5 isolates of *Psedomonas.ssp* (20%), Isolated *E.coli* by (4%), 4 isolates *Protuse.ssp* by (16%), 5 isolates *Klebsella.ssp* by (20%) as in Table 1-3.

Sub-corridor: Developing isolates included 27 isolates when different spores were initiated: 10 isolates of *Staph epidermis* (37.03%), 8 isolates of *Staph auras* (29.62%), 2 isolations of *E. coli* (7.4%), and 4 isolated *Psedomonas.ssp* (14.81%), 3 isolates of *Klebsella.ssp* (11.11) and isolated *Acenetobacter.ssp* (3.33%) as in Table 4-5. At the peak, the number of isolates was 30 isolates of different bacteria: 6 isolates of *Staph epidermis* (20%), 8 isolations of *Staph auras* (26.66%), 2 isolations of *E. coli* (6.66%), 6 isolates *Enterobacter.ssp* (20%), 7 isolated *Psedomonas.ssp* by (23.33%), and isolation of one *Klebsella.ssp* by (3.33%) as in Table 1-3.

Table 1.3: Species, numbers and percentages of bacterial isolates isolated from air pollution dishes at the start of work and in the midday.

The average	The third hall evening	The third hall is in the morning	The second surgical hall	The second surgery hall is the morning	The first surgical hall in the evening	First surgical hall in the morning	Sub corridor evening	Sub corridor Morning	The main corridor is evening	The main corridor is in the morning	Isolates
6.6	(%18.42) **7**	(%25.92) **7**	**6** (%16.21)	**6** (%22.22)	**10** (67.85%)	**7** (%26.92)	**6** (%20)	**10** (%37.03)	**2** (%8)	**5** (%20)	***Staph. Epidermidis***
9.3	(%42.1) **16**	**10** (%37.03)	(%32.43) **12**	**9 (%33.33)**	**9** (%32.14)	**6** (%23.07)	(%26.66) **8**	**8** (%29.62)	**5** (20%)	**10** (40%)	***Staph. Aureus***
2.6	**2** (%5.26)	**1** (3.70%)	**6** (%16.21)	**2** (%7.40)	**2** (%7.14)	**7** (%26.92)	**2** (%6.66)	**2** (%7.4)	**1** (%4)	**1** (%4)	***E. coli***
0.8	–	–	–	**1** (3.7%)	–	**1** (%3.84)	–	–	**4** (%16)	–	***Proteus spp.***
3.9	**7** (%18.42)	**2** (7.4%)	**3** (%8.10)	–	**5** (%17.85)	**1** (%3.84)	(%23.33) **7**	**4** (%14.81)	**5** (%20)	**5** (%20)	***Psedo.ssp***
1.6	**1** (%2.63)	**1** (%3.70)	**2** (%5.4)	**2** (%7.40)	_	**1** (%3.84)	**1** (%3.33)	**3** (%11.11)	**5** (%20)	–	***Klebsiella spp.***
4	**5** (%13.15)	**4** (%14.81)	(%21.62) **8**	**6** (22.22%)	**2** (%7.14)	**2** (%7.69)	**6** (%20)	_	**3** (%12)	**4** (%16)	***Entero. spp***
0.4	–	**2** (%7.4)	_	**1** (3.7%)	–	**1** (%3.84)	–	–	–	_	**Aceneto.ssp**
28.7	(%99.98) **38**	**27** (%99.96)	(%99.97) **37**	**27** (99.97%)	**28** (%99.98)	**26** (%99.96)	(%99.98) **27**	**27** (%96.27)	**25** (%100)	**25** (%100)	**Total**

There is an obvious difference in the increase in bacteria number when comparing the time of morning collection with the combination at midday, the daily average of bacterial species in the air operations halls from 0.4 to 9.3 per day, explain the fact that the number of people in the hall at midday more than their presence as these people become a natural source of airborne microorganisms, the diffusion of bacteria in the hall air depends on the quality of the ventilation. Poor ventilation leads to increase concentrations of microorganisms within the hall and environmental conditions, as well as the size of the hall.[10] Several bacterial isolates have been isolated in different operating halls, giving a clear indication of the possibility of a pandemic in the hospital, since the results do not belong to one bacterial type. Other advanced laboratory analysis such as field gel transfer technique and PCR should be performed to determine whether Bacterial isolates are descended from a single clone or multiple clone.[14][15] noted that some isolates of *S. epidermis* have the ability to produce a slime layer that helps them adhere to the surfaces of medical devices.[16] confirmed that the susceptibility of these bacteria to adhesion was an important means of infection. The results included the high prevalence of negative staphylococcus bacteria for coagulase testing in the operating halls as well as *S. auras* bacteria resistant to biosynthesis, nosocomial acquired infections, therefore conducting study is suggested which includes the study of the sensitivity of *S. auras* bacteria isolated from the air of the operating halls in line with the recommendations of the National Hospital Infection System (NNIS). These results were consistent with the results of[17], where the results showed that the most common bacteria in the halls were positive coagulase staphylococcus. While the results differed with the findings of[18] that the most common bacteria for patients with operating halls are *S. auras.*

The results of the study also showed the prevalence of Gram-negative Bacillus bacteria significantly in different sites of the halls as well as the corridors leading to the halls, if they were *Enterobacter*. Spp bacteria is the most common negative bacterial species of 4 daily. Followed by *Pseudomonas.* Spp bacteria is 3.9 times daily. A high percentage of pollution in the operating halls is also the main and secondary corridors, and may be due to its high prevalence of high resistance to multiple antibiotics and many disinfectants and disinfectants used in hospitals.[5] It is a source of infection for patients in the halls.[19] These bacteria are opportunistic pathogens, which need a few nutrients in their growth, and are difficult to completely eliminate due to their resistance to many antibiotics and chemical disinfectants because of their possession of the membrane and many factors of ferocity. Opportunist affects patients who have a defect or distortion in some of the defences of the immune

system, such as the treatment of immunosuppressant, such as in the case of transplantation or in the course of surgery, as well as when infected with HIV or other viral diseases, which lead to weak immunity of the body, taking advantage of this bacteria The weakness of the immune system leads to serious complications.[5]

The results also included prevalence *E.coli, Klepsella*.ssp and *Acenetobacter*. ssp (2.6, 1.6 and 0.4) respectively. The prevalence of these Gram-negative bacterial species is due to the possibility of remaining on the hands for 20 minutes. It is located between the folds of the skin and some types of resistance to detergents and disinfectants. Species are within the natural habitat of the human body.[20]

Pollution control swabs

The total number of swabs taken from the halls by the pollution control program was 135 when swabs were taken from different places including patient beds, floors, walls, anesthesia, surgical instruments and sterilizers. Twenty-seven isolates were isolated, with 17 isolation of human s*taphylococcus* isolates (62.96%), isolation *of E. coli* (11.11%), and isolation of *Bacillus.ssp* (14.81%) as in Table1.4.

Table 1.4: Number and percentage of developing isolates from pollution control swabs during the two months.

Growth rates	Isolates
(%62.96) 17	*Staph. Epidermidis*
(%14.81)4	*Bacillus*.ssp
(%11.11)3	*E. coli*
(88.88) 24	Total

We note from the above findings that the *staph epidermis* bacteria are the most popular in the Pollution Control programme surveys, with 62.96% of the total isolation of the pollution control program swabs, as the ratio was higher when comparing the results of the study with a local study conducted by the researcher.[21]

The isolation ratio was 22.95%, because the presence of *staph epidermis* in the current study was caused by the contamination of the medical staff in the basic class as well as leaving the door of the hall open for a long time helps to enter the air currents loaded with germs resulting from movement to the hall as well that the lack of good ventilation of the hall leads to an increase in the spread of bacteria. The presence of the *E. coli* bacteria is 11.11% as the

bacteria of the *E. coli* are included in the normal human skin, so it spreads on the floors, walls and exterior surfaces of the medical devices.[20]

The results of the statistical analysis using the ROC curves when comparing isolated bacterial species from the pollution control programme swabs with samples of microbial load showed that there was no correlation between bacterial isolates through sensitivity and specificity values as the sensitivity and specificity of the swabs with air models (4.43 and 2.5) respectively, the sensitivity means testED the positive result of the presence of bacteria in the air and specificity means the probability of having the same bacteria in pollution control program swabs explaining the value of the very weak specificity in the region under the curve of 2.5 is that there is no logical correlation between isolated isolates from two different sources as shown in Figure 1-1.

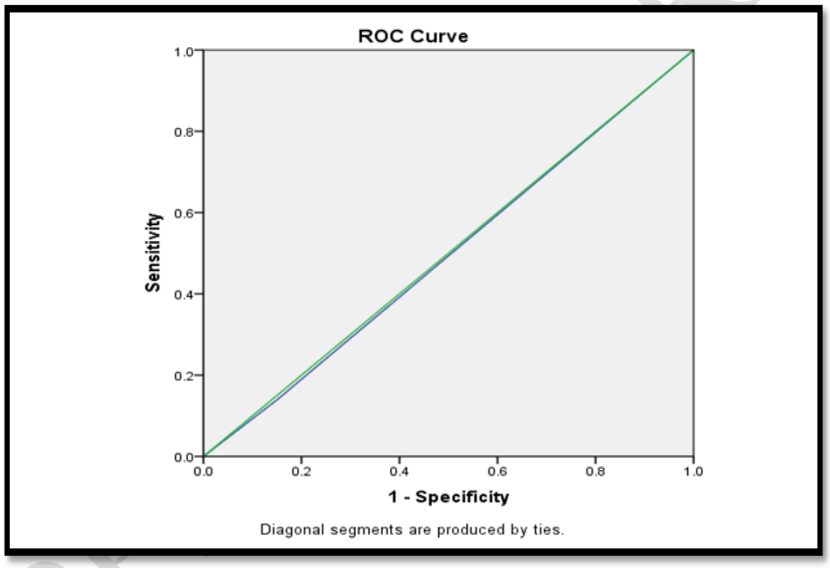

Figure 1-1: ROC curve for the sensitivity and specificity of the relationship between the pollutant control program swaps and the microbial load of air.

CONCLUSIONS

The microbiological quality of the air in the operating theaters varied significantly by the number of colony-forming units per cubic meter of air on the same day, When the average morning collection was compared with the mean at after noon. the average of the bacteria increased significantly, and this indicates the need to check the ventilation systems air filtration and recycling process, and this target to reduce the proportion of microbial load of ambient air that was within the limits of 228 CFU \ m3. The study also showed that the most common bacterial species that cause air contamination are that mesophilic and facultative

anaerobic bacteria, as well as pathogenic bacteria including *Staphylococcs*.ssp, *Pseudomonas*.ssp, *protuse*.ssp, *Enterobacter*.ssp and *E.coli*.

ACKNOWLEDGEMENTS

The authors would like to thank the staff (Doctors and Nurses) working at the hospital of Baquba City (Diyala Province), Iraq who have contributed in the sample collection.

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