



ISSN: 0067-2904

## Detection of Bacterial Population in Air Conditioner and Determine the Ability to Produce Biofilm

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### Abstract

There is not enough studies about bacterial contamination of air condition system in the cars and houses, bacterial detection of such surrounding is necessary for the human environment.

The object of recent study was to evaluate the level of bacterial contamination in air conditioner in cars and houses in Baghdad city, Iraq.

Air samples were taken indoor from cars and house air conditioner in the Baghdad city. The result indicated that gram positive bacteria more than gram negative bacteria in air conditioner. Air condition of cars (20-500 CFU) was more contaminated than of houses (10-100 CFU).

*Bacillus* was the most frequently bacterial isolates genus with recovery rate *Bacillus* spp.32%(10isolates) followed by *Staphylococcus epidermis* 16%(5 isolates), *Staphylococcus aureus* 16%(5 isolates), *Streptococcus pyogenes* 12.9%(4 isolates), *Klebsiella pneumoniae* 12.9%(4 isolates) and *Pseudomonas aeruogenosa* 9.6% (3 isolates).

The results of biofilm formation test by congored agar found that all bacterial species were biofilm producer.

**Keywords:** Air Conditioner, bacterial Population, cars

## التحري عن التجمعات البكتيرية في هواء المكيفات وتحديد قابليتها لانتاج الغشاء الحيوي

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### الخلاصة

لا توجد دراسات كافية عن التلوث البكتيري لانظمة التكييف في السيارات والبيوت. مراقبة التلوث البكتيري لهذه البيئات مهم لنوعية حياة الانسان. لذلك كان هدف هذه الدراسة هو تقييم انواع البكتريا الموجودة في هواء المكيفات. اخذت عينات هواء من تكييف السيارات والبيوت في مدينة بغداد، وجد ان البكتريا الموجهه لصبغة كرام اكثر من السالبة لصبغة كرام، وهواء التكييف للسيارات اكثر تلوثا (20-500)CFU منه في للبيوت (10-100CFU).

وجدت الانواع البكتيرية التالية :

*Bacillus* spp. 32%(10) عزلة *Staphylococcus epidermidis* 16%(5)

عزلة *Staphylococcus aureus* 16%(5) عزلة *Streptococcus pyogenes* 12.9%(4) عزلة

(عزلة) *Klebsiella pneumoniae* 12.9%(4) عزلة (3) *Pseudomonas aeruginosa* 9.6% (عزلة)

اظهرت نتائج اختبار تكوين الغشاء الحيوي باستخدام غراء احمر الكونكو ان جميع الانواع البكتيرية منتجة

للغشاء الحيوي .

## Introduction

There is similarity between air conditioning system and respiratory system in our bodies, this system supplies air to building and vehicles occupant. The respiratory system provides oxygenated air to the blood stream in a human body and is necessary to the human health. The significance of these two systems is important to humans [1].

There are two parts of air conditioning system : air handling unit and air duct is always wrap – enclose with material of fiberglass and other wrapping materials , air duct may also made of glass fiberboard [1].

Internal wrapping materials has a rough porous property of surface can catch particles and particulates found in the air . These trapped materials are : plant matter such as (decayed leaves, plant hairs, or fern spores), pollen grains, spores of fungi , parts of insect , paper fibers, other organic matter and skin chips [2,3].

These materials have hygroscopic properties that cause moisture absorption in air. Fungal spores can germinate and grow in this good moisture with accumulated dust. Many immunopathogenic cases were caused by these material such as allergies, infections, toxication and other symptoms called sick building syndrome [4, 5, 6].

The object of this search was to evaluate the content of bacterial contamination in airconditioner in cars and house in Baghdad, Iraq.

## Materials and Methods

Ten samples of cars air and ten samples of house air were collected from Al-dora and Al Jadyria in Baghdad city (during autumn) by using impaction method as described [7].

Microorganisms of air flow were impacted and directly collected in on nutrient agar, MacConkey agar, blood agar and Mannitol salt agar by putting plates in front of the conditioning vents after the system had been running for 2min, the distance between air outlets of air conditioning and plate was 15 cm. Each sample took 3 min.

Samples were inoculated then incubate for 48 hr at 37 °C, after incubation period bacteria colonies were enumerated. Morphological properties of isolates were tested by gram stain, biochemical tests (indole, catalase, oxidase and methyl red -Voges prokauer ) and for further identification , vitek system were used .

Congo red agar was used for biofilm formation test, this medium was prepared by melting 37g of brain heart infusion broth, 50g of sucrose and 15g of agar-agar in 900 ml of D.W. sterilized, cooling to 55°C, added 100ml of Congo red solution (0.8 %) then poured into Sterilized petri-dish.

Inoculation of this medium with single colony by streaking and incubation at 37 °C for 48 hr ,positive result ( biofilm producer ) is black colonies while negative result( non biofilm producer ) is pink colonies [8].

## Results and Discussions

Results revealed that total of six morphological different bacterial species were isolated among which gram positive and negative stain ,according to diagnosis result by the microscopic and morphological characteristics and vitek system, *Bacillus* was the most frequently isolated bacterial genus with recovery rate *Bacillus* spp. 32%(10 isolates) followed by *Staphylococcus epidermidis* 16%(5 isolates), *Staphylococcus aureus* 16%(5 isolates), *Streptococcus pyogenes* 12.9%(4 isolates) ,*Klebsiella pneumoniae* 12.9%(4 isolates) and *Pseudomonas aeruginosa* 9.6% ( 3 isolates ) Tables-(1, 2) .Figures-(1, 2, 3, 4, 5)

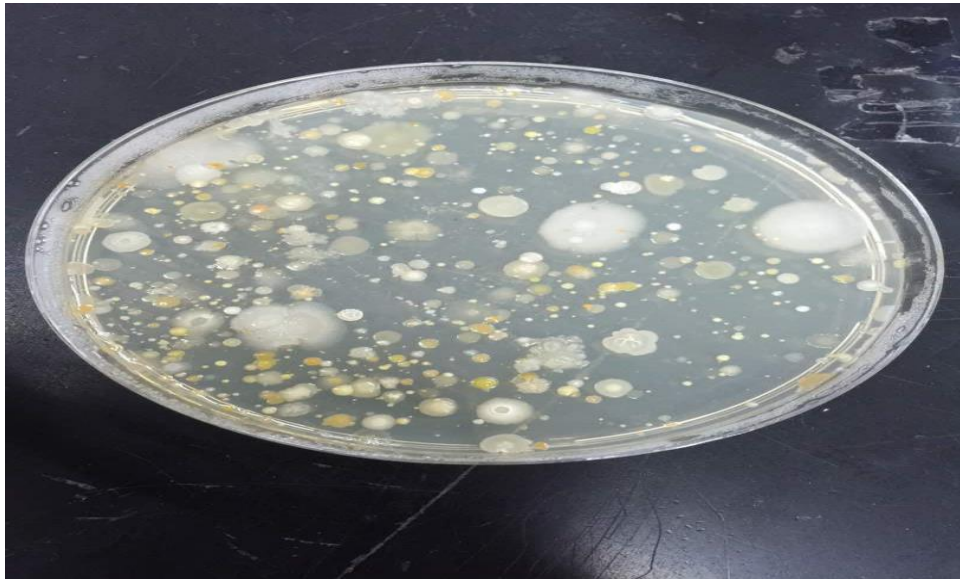
**Table 1-**Bacterial species isolated from air conditioners

Bacterial species	%(No.)
<i>Bacillus spp</i>	32%(10 isolates)
<i>Staphylococcus epidermidis</i>	16%(5 isolates)
<i>Staphylococcus aureus</i>	16%(5 isolates)
<i>Streptococcus pyogenes</i>	12.9%(4 isolates)
<i>Klebsiella pneumoniae</i>	12.9%(4 isolates)
<i>Pseudomonas aeruginosa</i>	9.6%(3 isolates)

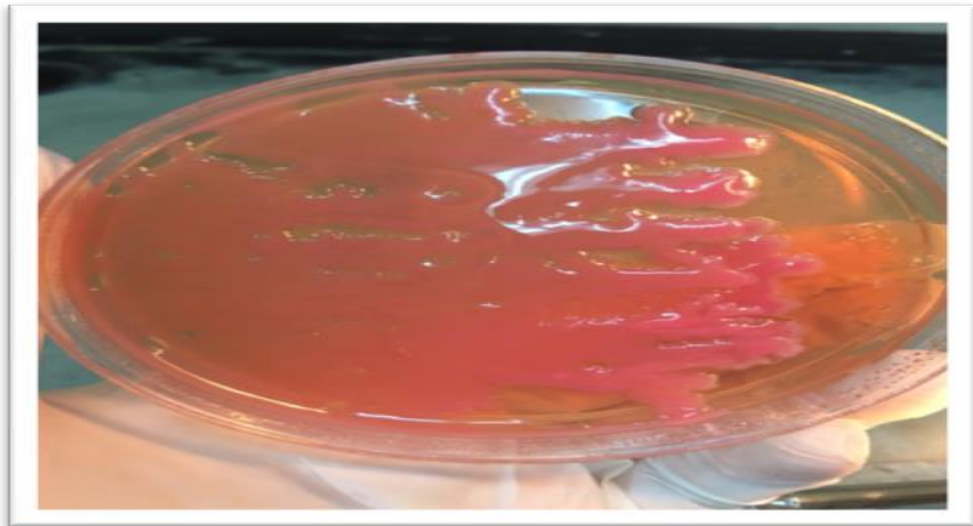
**Table 2-**Prevalence of bacterial spp. in air of cars and houses conditioners

Bacterial spp	Bacterial isolats % (cars)	Bacterial isolats % (Houses)	Total
<i>Bacillus spp</i>	8(80%)	2(20%)	10
<i>Staphylococcus epidermidis</i>	4(80%)	1 (20%)	5
<i>Staphylococcus aureus</i>	3(60%)	2(40%)	5
<i>Streptococcus pyogenes</i>	2(50%)	2(50%)	4
<i>Klebsiella pneumoniae</i>	2(50%)	2(50%)	4
<i>Pseudomonas aeruginosa</i>	3(100%)	0 (0%)	3

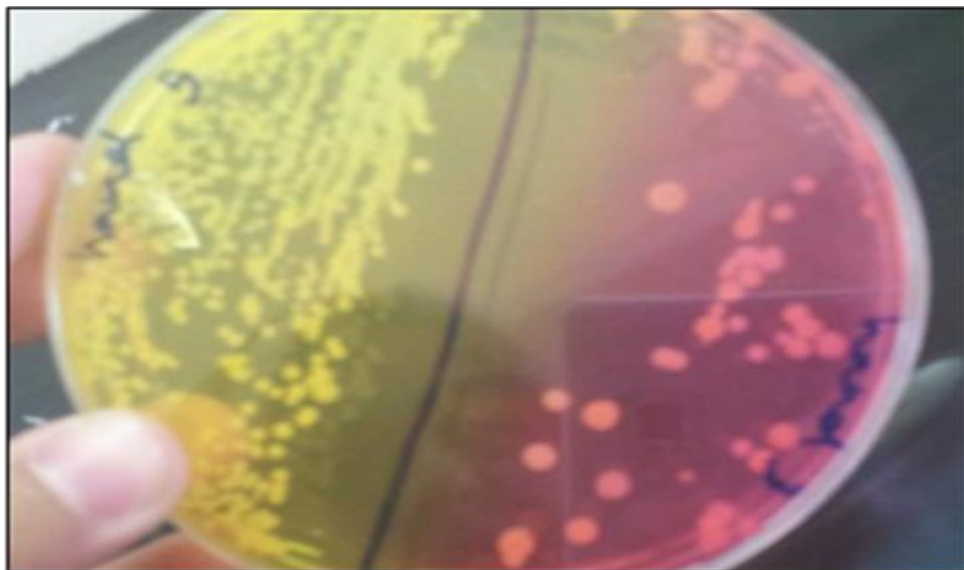
**Figure 1-**acterial colonies isolated from air conditioners of house on nutrient agar



**Figure 2-**Bacterial colonies isolated from air conditioners of cars on nutrient agar



**Figure 3 - e** on macConky agar *Klebsiella pneumonia*



**Figure 4 -** *Staphylococcus aureus* *Staphylococcus epidermidis* on manitol salt agar

Results of recent study agreed with previous study [9] who found that air conditioner contaminated with *Staphylococcus aureus* and *Streptococcus pyogenes*, but not agreed with AIMjati [10] who found that gram negative bacteria had more recovery rate than gram positive bacteria, previous researchers [11] isolated *Bacillus spp* from air conditioners that in agreement with recent study.

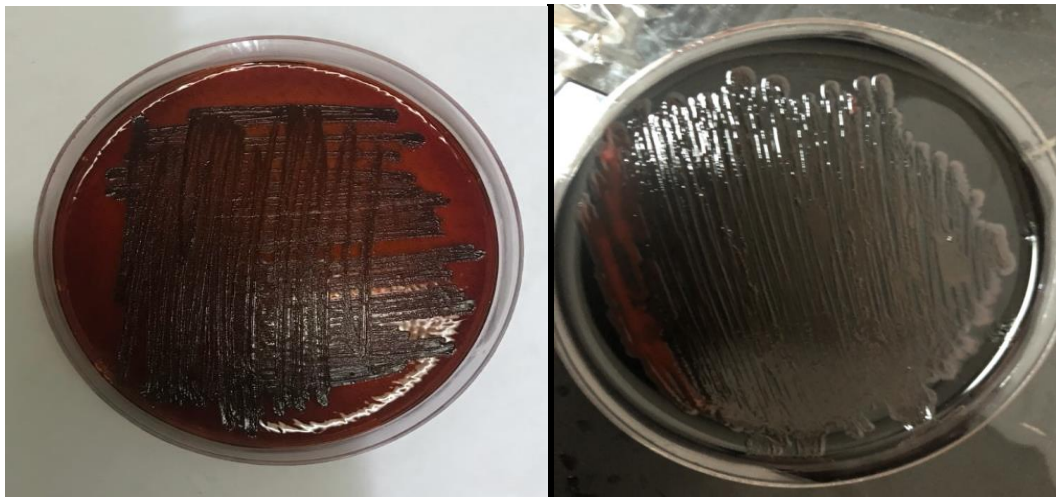
The colony forming unite CFU of air conditioners in cars was more than CFU of houses

That may due to small size of cars cabin compared to houses that very harmful because passengers will be able to inhale bacteria through inhaling airborne particles, this problem considered as one of indoor pollution type [12].

Bacterial cell number varied between (10-100) CFU in air of houses while (20-500) CFU in air of cars. The cause of these bacterial proliferation is dust accumulation coupled with humidity these microorganisms capable of surviving the prevailing conditions [13].

Bacterial contaminants of air conditioner act as proteins with allergenic nature, toxins (endotoxins in especial). Musty odors is the result of the contaminated air conditioner system in building that mean microbial growing occurred in the system, opportunistic infections may caused by *P. aeruginosa* [5,6].

Recent results of biofilm formation test by congo red agar found that all bacterial species were biofilm producer (100%) (gave black colonies on congo red agar), black color of colonies due to binding of congo red with exopolysaccharides [8].



**Figure 5-** Biofilm producer bacteria on congo red

There are several immunopathogenic disorders caused by inhalation of bacteria like bacterial infections, allergies, and toxic reactions [4,5], also sick house syndrome with symptoms include headache, watery eyes, skin disorders and weakness [6].

There are many methods that decrease level of bio-contamination in the system of air conditioning there are:

1. Improve of filtration effectiveness of the system. Replacement and cleaning should be done According to the manufacturer's instructions
2. Regular cleaning and maintenance of the cooling coil and drainage container 2-4 times a year that depend on the age, operation, history and uses of the system.
3. The air handling unit and the duct should has good insulation to decrease microbial growth and biocontaminants accumulation, that achieved by improve of filters, make sure that filters are installed correctly, filter changing (seasonably) and cleaning the drainage containers and insulation material.
4. Periodic cleaning of air conditioning system when there is visible indication of microbial growth and accumulation of heavy dust, asking professional staff for evaluation.
5. The air conditioning systems were designed without occurring of air contact internal insulation with a rough porous nature of surface.
6. Air intakes of air conditioning system must be farther road, cooling tower and loading port [14].

## Conclusion

The results of recent study led to the suggestion that the air condition is source of pathogenic bacteria such as *Bacillus*, *S. epidermidis*, *S. aureus*, *S. pyogenes*, *K. pneumoniae* and *P. aeruginosa*, all these species were biofilm producers.

## Reference

1. Yang, C. S. **2000**. *Biologist contamination HVAC system*. issue microbiology services in cherry Hill, Newjersey.
2. Holmer, I., Nilsson, H., Bohm, M. and Noren, O. **1995**. Thermal aspects of vehicle comfort. *Appl Human Sci*, **14**: 159-165.
3. Ahearn, D.G., Crow, S.A., Simmons, R.B., Price, D.L., Noble, J.A. and Pierson, D.L. **1996**. Fungal Colonization of Fiberglass Insulation in The Air Distribution System of A Multi-Story Office Building VOC production and possible relationship to sick building syndrome. *J Ind Microbiol*, **16**: 280-285.
4. Simmons, R.B., Rose, L.J., Crow, S.A. and Ahearn, D.G. **1999**. The occurrence and persistence of mixed biofilms in automobile air conditioning systems. *Curr Microbiol*, **39**: 141-145.
5. Kumar, P., Marier, R. and leech, S.H. **1981**. Hypersensitivity pneumoitis due to car air conditioner. *N Engl.J.Med*, **305**: 1531-1532.
6. Kumar, P., Marier, R. and leech, S.H. **1984**. Respiratory allergies Related to Automobile Air Conditioners *N Engl.J.Med* **311**: 1619-1621
7. Meyer, G. and Schepers, B. **2007**. Does air conditioning impact on hygienic quality of indoor air on seagoing vessels, *Internat. Marit. Health*, **58**: 1-4
8. Freeman, D.J., Falkiner FR and Keane CT. **1989**. New method for detecting slime production by coagulase negative staphylococci, *J. Clin. Pathol.* **42**: 872-874.
9. Genet C, Kibra, G. and Tsegaye, W. **2011**. Indoor air bacterial load and antibiotic susceptibility pattern of isolation operating room south west Ethiopia voi.zi, No.1, *Ethiopia.J.healthsei*
10. Al-Mijalli, S.H. **2016**. Bacterial Contamination of Indoor Air in Schools of Riyadh, *Saudi Arabia HS, Air Water Borne Dis*, 2016, **6**: 1
11. Anas, G., Aligh, D.S., Suleiman, G. and Warodi, F. A. **2016**. Studies on Microorganisms Associated with Air-Conditioned Environments. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR)*, **10**(7): 16-18
12. Górný RL, Dutkiewicz J, Krysińska- Traczyk E. **1999**. Size distribution of bacterial and fungal bioaerosols in indoor air *Ann Agric Environ Med.* **6**: 105-13.
13. Nordel, E.A. **2000**. Contamination of air conditioners filter. *American review of respiratory diseases*, : 501-503.
14. Owen, MK., Ensor DS, Sparks LE. **1992**. Airborne particle sizes and sources found in indoor air. *Atmos Environ.* **26**: 2149-62.