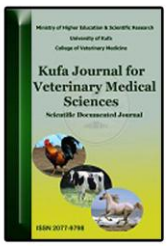
Kufa Journal for Veterinary Medical Sciences Vol.4 No.1 (2013) 81-89

Kufa Journal for Veterinary Medical Sciences
www.vet.kufauniv.com



Comparative Study of Bacteria and fungi air polluted Slaughterhouse of Al-Diwaniya City

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Abstract:

This research was studied for the period between November 2012 – February 2013. which included compared the numbers and species of bacteria and fungi found in the air inside and around the slaughterhouse. The results of statistical analysis show significant differences between the numbers of bacterial colonies isolate of air between inside and around the slaughterhouse during the months of study especially during the first month of the study and during the second month for the fungal colonies with no significant differences between the numbers of colonies isolate of air inside and outside the slaughterhouse . Study proved that total numbers in the air was about 196 bacterial colony and 22 fungal colony for environment inside slaughterhouse and 266 bacterial colony and 43 fungal colony for environment around the slaughterhouse. The results showed that eight types of a bacteria was isolated : *Staphylococcus aureus,* formed highest appearance frequency (16.68%) in both inside and around the slaughterhouse, *E.coli* (16.64%), *Streptococcus pyogenes* (9.69%) , *Bacillus subtilis*(7.55%), *Salmonella enterica* (3.83%) , *Enterococcus faecalis*(15%), *Klebsiella pneumoniae* (14.09%) , *Pseudomonas aeruginosa* (16.34%), and isolated and diagnosed five types of fungi from the air around the slaughterhouse which are: *Aspergillus flavus, Asper. niger, Mucor sp., Pencillium notatum, Pencillium sp*., and four types of fungi inside slaughterhouse expect *Asper. niger*, and was the highest appearance and frequency of the fungi in the inside and around *Pencillium notatum* (26.25%). This evidence of air pollution, the bacteria and fungi, have an important role both in spreading of diseases for humans and animals by the contamination of meat and in addition that are handled within the slaughterhouse and therefore dangerous to the health of the consumer to that air polluted in and around the slaughterhouse.

Key words: slaughterhouse**,** bacteria**,** fungi**,** contamination

دراسة مقارنة لبكتريا وفطريات الهواء الجوي لمجزرة الديوانية

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الخلاصة:

صمم هذا البحث خلال مدة ثلاثة شهور للفترة من 1\11\2012 – 1\2\2013 ، و تضمن مقارنة اعداد وانواع البكتريا والفطريات المحمولة في الهواء الجوي داخل وحول مجزرة الديوانية، اوضحت نتائج التحليل الاحصائي وجود فروق

معنوية بين اعداد المستعمرات البكتيرية المعزولة من الهواء داخل وحول المجزرة خلال اشهر الدراسة وخاصة خلال الشهر الاول و الشهر الثاني للدراسة بالنسبة للمستعمرات الفطرية مع عدم وجود فروقا معنوية بين اعداد المستعمرات المعزولة من الهواء داخل وحول المجزرة . اثبتت الدراسة ان معدل اعداد البكتريا الموجودة في هواء المجزرة كان بحوالي 196 مستعمرة بكتيرية للبيئة داخل المجزرة و 266 مستعمرة بكتيرية للبيئة حول المجزرة ، و22 مستعمرة فطرية للبيئة داخل المجزرة و43 مستعمرة فطرية للبيئة حول المجزرة ، كما اظهرت النتائج عزل وتشخيص ثمانية انواع بكتيرية وهي *Staphylococcus aureus* والتي شكلت اعلى نسبة ظهور وتردد في داخل وخارج المجزرة (16,68 % ، *Streptococcus pyogenes* (9,69)% ، *Bacillus subtilis* (7,55)% ، *Salmonella enterica* (3,83)% ، *E. coli* (16,46)% ، *Enterococcus faecalis* (15)% ، *Klebsiella pneumoniae* (14,09) ، كما تم عزل وتشخيص خمسة انواع فطرية من الهواء حول المجزرة وهي *Aspergillus flavus* ، *Asper. niger* ، *Mucor sp.* ، *Pencillium notatum* ، *Pencillium* sp. واربعة انواع فطرية من الهواء داخل المجزرة وهي *Aspergillus flavus* ، *Mucor sp.* ، *Pencillium notatum* ، *Pencillium* sp.، وكان اعلى نسبة ظهور وتردد للفطريات في داخل وحول المجزرة هي للفطر *Pencillium notatum* (26,25 %). يعتبر هذا البحث دليلا على تلوث الهواء بالبكتريا والفطريات المسببة للامراض في الانسان والحيوان نتيجة تلوث اللحوم التي يتم التعامل معها داخل المجزرة وبالتالي تكمن خطورتها على صحة المستهلك.

Introduction:

The subject of air pollution became on the most important topics, where enacted most countries of the world multiple laws in order to maintain the cleanliness of the air, and prevention of pollution (1). In Iraq the slaughterhouse is one of the areas that have been exposed to biological contamination. Workers and the carcasses is in contact with thousands of cubic meters of air per day, which carry dust and plankton solid bearer to revive microorganisms cause many diseases for human, including allergies, respiratory infections, and contamined the carcasses, and from the air was isolated 19716 fungal strain from 533 sample and these fungi change its rate in the air with different seasons , While the number of airborne bacteria are very high (2). Influenza virus spread in the air, it's the more viruses. and the microbes also used in the germ wars for its ease development in the air, and cause deadly diseases to humans and most famous of these microbes in the present is cause of anthrax which caused by *Bacillus anthracis* which can spread by the air, as the plague (Black pasturella pestis plague) and smallpox which is caused by smallpox virus (3). The most important reasons that lead to air pollution in the slaughterhouse is bad dealing with the animals that enter the slaughterhouse for the purpose of slaughter , hand health screening and isolation until sure of their safety and suitability for human use, also due to wrong dealing with the waste carcass properly. The serious of air pollution ranging according to the type and degree of pollution and areas of presence (4).Air pollution produces with bacteria, fungi, and molds from decomposition of plants and animals and waste that lead to economic loss in the world each year (Journal of environment,2000) . In the air, spread many types of bacteria and fungi in the case of static state, and humans infected if given the appropriate conditions. The bacterial genera: ***Mycobacterium, Streptococcus, Corynebactrium, Yersina,*** **and fungi** ***Candida, Pencillium, Aspergillus*** is spreading in air (3)**.**

Because lack of studies on bacteria and fungi associated with air of the slaughterhouse of Diwaniya this research came for the purpose of disclosure attributed the prevalence of certain bacteria and fungi contaminated the air environment of Diwaniya slaughterhouse.

Material and methods:

Disposables Sterile plastic Petri dishes which used contain media of Potato Dextrose Agar (PDA)or Sabouraud

dextrose agar (SDA) for the cultivation and isolation of fungi, and Petri dishes containing nutrients media, Nutrient Agar, Blood Agar, Manitol Salt Agar, Simmon Citrate Agar, Urease Agar, MacConkey Agar, produced by (Himedia Company) for culture and isolation of bacteria. Selected two regions of Al- Diwaniya slaughterhouse (inside and around the slaughterhouse) with several places , took (25) sample of each regions and the total samples (50) sample each month and the number of total samples (150) sample, dishes taken to each place of each region then presented dishes to the outer air for the purpose of contaminated by air by opening the dishes for (10) minutes ,after that, dishes was closes and placed in plastic bags ,then transferred to the laboratory. .

Sampling process continued for a period of three months, from (01.11.2012 (1st), 01.12.2012 (2^{nd}) to 01.02.2013 (3^{rd})months , every month is transferring samples and grow and isolate microorganisms, incubated dishes containing media PDA or SDA at a temperature of 25 C for ten days. The dishes containing nutrient media for bacteria incubated at a temperature 37 ° C for 24 hours and examined dishes to make sure there growth or lack of it. Diagnosed isolated fungi depending to (5) and, (6) and (7). species of bacterial isolates Diagnosed by prepared swabs of bacteria isolated on Nutrient Agar and then stained by gram ′s stain, and examined under the microscope using lens oily (100 X) , biochemical tests done to the grow cultures for the purpose of diagnosis (8), and depending on (9), (10), (11), and (12), It was also interesting to note the external appearance of the colonies in terms of color, size and shape of the growth.

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Results:

The results show that air samples within slaughterhouse is gave the positive result for isolating of bacteria (100%), and the total number of aerobic bacteria count for the period from the beginning of November until the end of January is (426) colony, where was the number of colonies inside the slaughterhouse (196) bacterial colony, and around slaughterhouse (266) bacterial colony,. Was conducted of independence chi square between the three measurements of bacteria (according to the periods 1^{st}, 2^{nd}, 3^{rd} month) for each Inside and around the slaughterhouse shows that it is clear that no significant differences. But to see the difference between bacteria for each month alone between inside and around the slaughterhouse there is significant differences between inside and around the slaughterhouse in the first month; Figure (1) shows that

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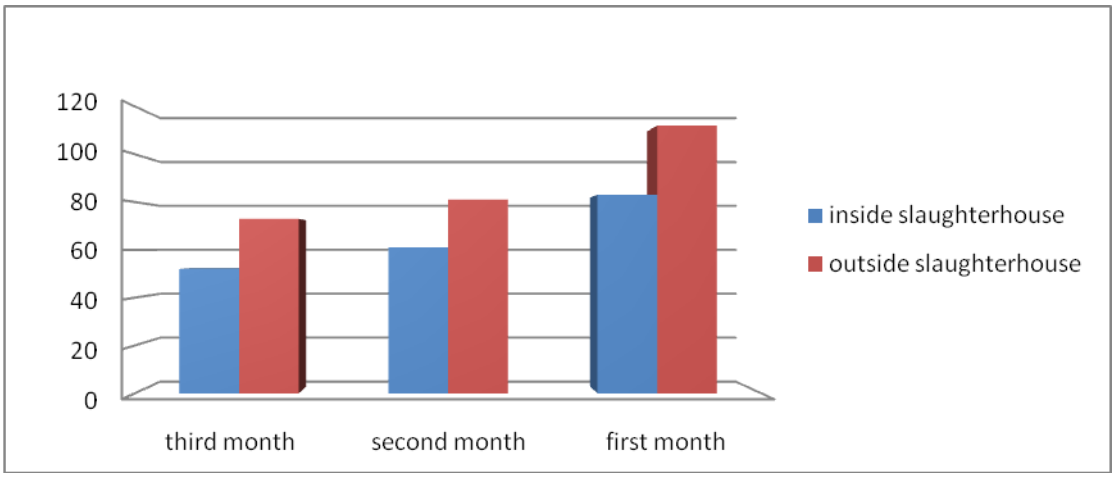

Figure (1): **average of bacterial colonies according to place of the slaughterhouse**

while did not give all the samples as a positive result to fungal isolate ,where the overall rate for the preparation of fungi isolated from the air and from the same period (65) fungal colonies: (22) fungal colony of the air inside the slaughterhouse and (43) fungal colonies from the air around slaughterhouse. Was conducted of independence chi square between the three measurements of fungi (according to the periods 1st, 2nd, 3rd month) for each Inside and around the slaughterhouse shows that it is clear that no significant differences. But to see the difference between fungi for each month alone between inside and around the slaughterhouse there is significant differences between inside and around the slaughterhouse in the second month. Figure (2) shows that.

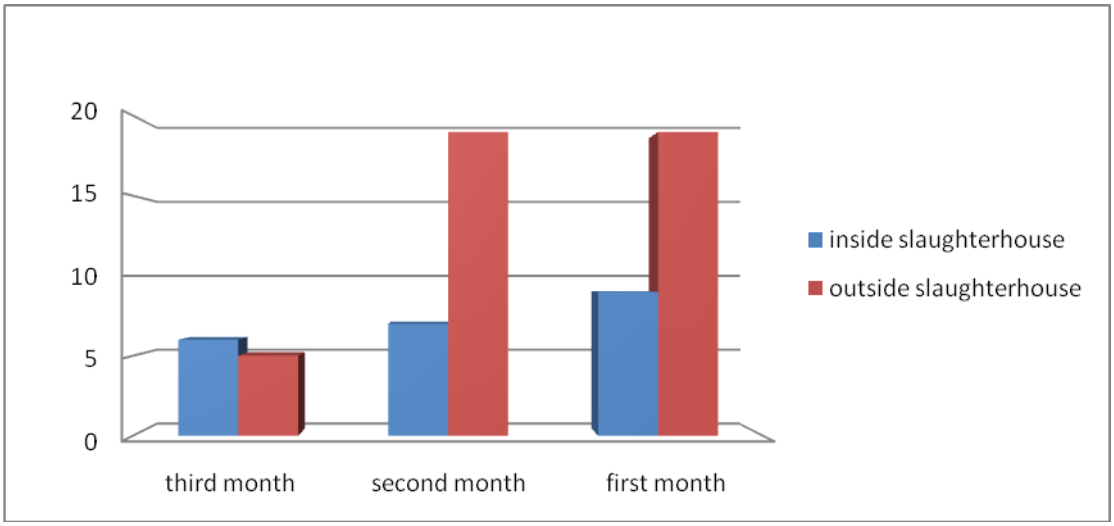

Figure (2): **average of fungal colonies according to the place of the slaughterhouse**

But to see the difference between the bacteria inside and around of the slaughterhouse there were significant differences either for fungi there are not any significant differences as well as there are no differences between the four indicators of bacteria and fungi inside and around the slaughterhouse. Figure (3) shows that.

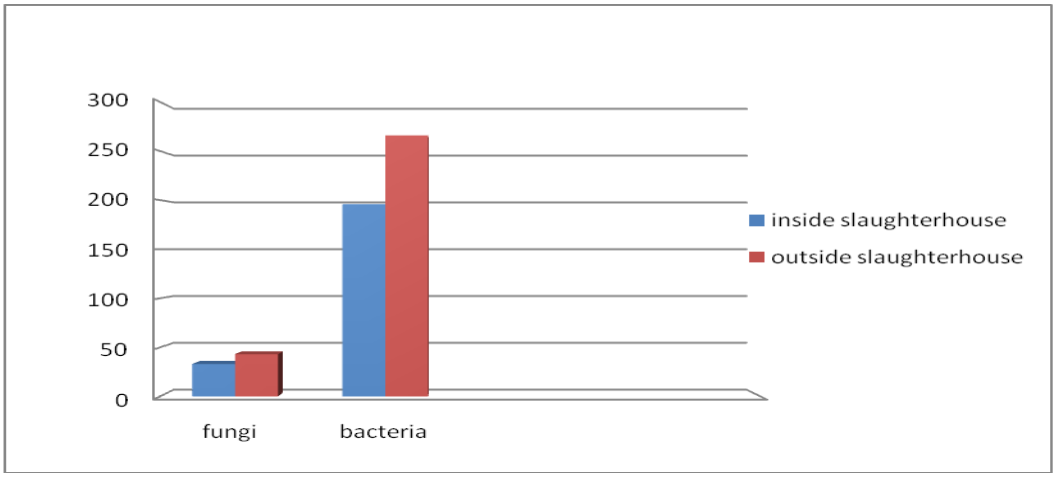

Figure (3): **Comparison between bacterial colonies and fungal colonies according to place of the slaughterhouse**

Depending on the phenotypic qualities and biochemical tests was isolated and diagnosed eight different types of bacteria from air samples of air inside and around the slaughterhouse, which totaled of its Isolates (439) bacterial isolates from air inside slaughterhouse ,and (488) bacterial isolates from air around slaughterhouse, that the highest proportion of bacteria have been isolated from the air of slaughterhouse is the bacteria *Staphylococcus aureus* (148 isolates: 75 isolates of the air inside the slaughterhouse by 17.1% and 73 isolated from the air around the slaughterhouse by 16.3%), followed by bacteria *E.coli* (146 isolates :75 isolated from the air inside the slaughterhouse by 17.1% and 71 isolated from the air around the slaughterhouse by 16%) , and bacteria *Ps. aeruginosa* (145 isolates: 73 isolates from the air inside the slaughterhouse massacre by 16.62% and 72 isolates from the air around the slaughterhouse of 16%) , *Enterococcus faecalis* (133 isolates: 68 isolates from the air inside the slaughterhouse massacre by 15.5% and 65 isolates from the air around the slaughterhouse of 14.5%) , *Klebsiella pneumoniae* (125 isolates: 66 isolates from the air inside the slaughterhouse massacre by 15% and 59 isolates from the air around the slaughterhouse of 13.2%) , *Streptococcus pyogenes* (86 isolates: 54 isolates from the air inside the slaughterhouse massacre by 10.25% and 41 isolates from the air around the slaughterhouse of 9%) , *Bacillus subtilis* (70 isolates: 19 isolates from the air inside the slaughterhouse massacre by 4.32% and 51 isolates from the air around the slaughterhouse of 11.4%) , *Salmonella enterica* (34 isolates: 18 isolates from the air inside the slaughterhouse by 4.1% and 16 isolates from the air around the slaughterhouse of 3.6%). Through statistical analysis results show there is no significant difference between the numbers of bacterial isolates for inside and around the slaughterhouse a table (1). .

Table (1): genera and species of isolated bacteria: * Significance

Bacterial isolates	**place**				**No. isolates**	Chi square value
	Inside slaughterhouse		**around slaughterhouse**			
	No.	**%**	**No.**	**%**		
Staphylococcus aureus	75	17.1	73	16.3	148	0.027027
Streptococcus pyogenes	45	10.25	41	9	86	0.186047
Bacillus subtilis	19	4.32	51	11.4	70	14.62857*
Salmonella enteric	18	4.1	16	3.6	34	0.117647
Escherichia coli	75	17.1	71	16	146	0.109589
Enterococcus faecalis	68	15.5	65	14.5	133	0.067669
Klebsiella pneumoniae	66	15	59	13.2	125	0.392
Pseudomonas aeruginosa	73	16.62	72	16	145	0.006897
Total	**439**	**100**	**448**	**100**	**887**	0.091319

The bacteria have been diagnosed with this bacterial isolates through phenotypic, culturing and biochemical description for all bacterial isolates contaminated air inside and around the slaughterhouse.

- For isolating of fungi : We have been isolated fungi using media Sabouraud dextrose agar and potato dextrose Agar and fungi diagnosed depending on the color , diameter and shape of the colony on the dish, and a microscopic examination using lactophenol cotton blue dye to identify the form of conides which is a diagnostic test of fungi type . the results showed that the highest proportion of frequency in the air is *Pencillium notatum* (21 isolates: 6 isolates from the air inside the slaughterhouse by 26.1% and 15 isolates from the air around the slaughterhouse by 26.3%), followed by fungus *Aspergillus niger* (20 isolates from the air around slaughterhouse only 35.1%), followed by fungus *Pencillium sp*. (15 isolates: 6 isolates of the air inside the slaughterhouse by 26.1% and 9 isolates from the air around the slaughterhouse by 15.78%), fungus *Mucor SP*. (13 isolates: 7 isolates of the air inside the slaughterhouse by 30.4% and 6 isolates from the air around the slaughterhouse by 10.52%, and fungus *A. Flavus* (11 isolates: 4 isolation of the air inside the slaughterhouse by 17.4% and 7 isolates from the air around the slaughterhouse by 12.3%, as the total number of isolates (80) isolate. table (2).

Table (2): genera and species of isolated fungi

Fungal isolates	place				No. isolates	Chi square value
	inside slaughterhouse		around slaughterhouse			
	No.	%	No.	%		
Aspergillus flavus	4	17.4	7	12.3	11	0.818182
Aspergillus niger	-	-	20	35.1	20	20*
Mucor sp.	7	30.4	6	10.52	13	0.076923
Pencillium notatum	6	26.1	15	26.3	21	3.857143*
Pencillium sp.	6	26.1	9	15.78	15	0.6
total	23	100	57	100	80	14.45*

* Significance

The Discussion:

In this study, isolate and diagnose many genera and species of bacteria and fungi which contaminated air inside and around of the slaughterhouse in Al-Diwaniya city, and these microbes are the primary cause of acute and chronic food poisoning disease. As shown by this study that the bacteria negative for gram′ s stain was most common in the air pollution inside and around the slaughterhouse, with a total isolates (583) isolates, while the number of bacterial isolates positive for gram′ s stain (304) isolates back to bacteria *S.aureus* , *Strep* . *Pyogenes* and *Bacillus subtilis.* table (1), That the reason for the prevalence of bacteria *S.aureus* is Due to it has many of the mechanisms (resistance surface antigen) , and produces certain enzymes or extracellular material, and some are internal toxins which heat resistance its be a very dangerous source (13), and that many of them carries by plasmids.. As well as the advantage of its ability to grow in variable environmental conditions of temperature and pH (14). As for the bacteria *E.coli* are bacteria widespread in the environment, water and food pollution the main source of spread it,

and its presence indication for direct or indirect contamination with fecal matter (15), (16); (17). The reason for the existence of *Ps.aeruginosa* attributable to provide appropriate nutrients for their growth and to it has pigments which have an important role in the granting of these bacteria strength to compete with the other types of bacteria in place where it's found, as these pigments has similar activity to do antibiotics which leads to inhibition of these other types which present with it And have the opportunity to sovereignty (18) (19).

This explains the fact that the Meat is contaminated often with these bacterial species, according to a study (20) and study (21); (22), where explained that food of animal origin (meat, for example), whether cooked or not they are contaminated with two kinds of bacteria, in particular the two *S.aureus & E.coli*. Through these results show that the prevailing rate was to *S.aureus & E.coli* where these germs are natural flora found in humans and animals, but their presence in food is a sign of unhealthy deal through processing (23). The results of this study also show that genus *Pencillium* is the most presence in its high number of colonies and to the most places that have been isolating inside slaughterhouse during the study period, may explain the widespread this fungus as is often thought in the air and grow on waste and organic waste to increase the breeding and spread in the environment (24) and producing conidial spores dense in number is transmitted by air, and this in turn also applies to the presence of other species of the fungi, which occupied the forefront of the fungi that have been isolated from the air, which were represented by *Aspergillus sp. , Mucor sp.* , Where the reason for the prevalence of fungus *Aspergillus sp.* to being one of the fungi that features produce large amounts of spores , small in size, which remain suspended in the environment which they are located for long periods, as well as possession of multiple enzymes analyze for protein and cellulose materials, as well as the right conditions for growth and reproduction like moisture and temperature and are available in the internal environment of the slaughterhouse. Where This study showed the presence of many common fungal genera and species and that found in the air, where fungal growth increased markedly by increased organic matter because its use it as an energy source (25), and that the source may be a result of contamination of the environment and the circumstances surrounding the slaughter of animals, and this agree with (26), which pointed to air pollution in Al-Diwaniya city of this fungus, on the grounds that the air of the most important sources of pollution. .

This study concludes that there is no significant difference between the numbers of bacteria as well as fungi of the air inside the slaughterhouse during the months of study and also the case of the air around the slaughterhouse, either when comparing numbers of bacteria by months of research between the air inside and around the slaughterhouse turned significant differences between the air inside and around the slaughterhouse during the first month, and during the second month for the fungi present in the air between the inside air and around the slaughterhouse, and there were significant differences between the total numbers of bacteria present in the air of the environment inside and around the slaughterhouse either fungi There has not been any significant differences.

Acknowledgements

Extend my thanks and gratitude to the veterinarian (Azhar Ali) for continuing to offer help to me.

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