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A STUDY ON THE EFFECT OF GARLIC AND *NIGELLA SATIVA* ON SOME FOOD POISONING BACTERIA ISOLATED FROM READY-TO-EAT MEAT SANDWICHES IN ALEXANDRIA CITY

(With 7 Tables)

By

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دراسة تأ ثير الثوم وحبة البركه على بعض بكتيريا التسمم الغذائى المعزوله من سندوتشا ت اللحوم الجاهزه فى مدينة الأ سكندريه

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تم تجميع مائة عينه عشوائيه من سندوتشات السجق، البيف برجر، الشاورمه والكبده الجاهزه للأكل (25 من كل نوع) من بعض المطاعم والباعه الجائلين بمدينة الأسكندريه وذلك بهدف التعرف على الحاله البكتيريه لها من حيث العد الكلى للبكتيريا الهوائيه والعد الكلى لكل من ميكروب عصيات سيريس، السبحى البرازى والمكورالعنقودى الذهبى حيث أظهرت النتائج ان متوسط العد الكلى فى سندوتشات السجق، البيف برجر، الشاورمه والكبده كان ($16 \pm 3.2 \times 10^{4}$)، ($2.3 \pm 1.2 \times 10^{4}$) ، ($4.2 \pm 2.1 \times 10^{4}$) و ($3.6 \pm 1.8 \times 10^{3}$) خليه/ جم بالترتيب وقد ثبت وجود فروق معنويه بين هذه المتوسطات وكانت نسبة العينات الموجبه لميكروب عصيات سيريس (100 ، 92 ، 84 ، 72) % و بمتوسطات ($2.75 \pm 1.4 \times 10^{4}$)، ($2.4 \pm 1.2 \times 10^{2}$)، (35.2 ± 17.6) و ($3.7 \pm 1.8 \times 10^{3}$) خليه /جم على التوالى. وقد كانت هناك فروق معنويه بين سندوتشات السجق والكبده من ناحيه وكل من البيف برجر، الشاورمه. وكانت نسبة العينات الموجبه لميكروب السبحى البرازى (88 ، 84 ، 88 ، 76) % بمتوسطات ($45.7 \pm 5.9 \times 10^{3}$) ، ($28 \pm 7 \times 10^{2}$) ، ($3.1 \pm 1.5 \times 10^{2}$) و ($2 \pm 0.6 \times 10^{3}$) خليه/جم. وقد كانت هناك فروق معنويه بين سندوتشات السجق والبيف برجر من ناحيه وكل من الكبده والشاورمه. وكانت نسبة العينات الموجبه لميكروب المكورالعنقودى الذهبى (92 ، 80 ، 88 ، 80) % و بمتوسطات ($3.25 \pm 1.6 \times 10^{3}$)، ($2.8 \pm 1.4 \times 10^{2}$)، ($4.1 \pm 2 \times 10^{3}$) و ($4.8 \pm 2.4 \times 10^{3}$) خليه/جم لكل من سندوتشات السجق، البيف برجر، الشاورمه والكبده

على التوالى. وقد كانت هناك فروق معنويه بين سندوتشات الكبده والشاورمه من ناحيه وكل من البيف برجر والسجق. وقد تم كذلك دراسة تركيزات مختلفه من الثوم المهروس طازجا بتركيز (2 ، 4 ، 6) % وكذلك حبة البركه المطحونه بتركيز (1 ، 3 ، 5) % مع اللحم المفروم ضد الثلاث ميكروبات المذكوره سابقا عند درجة حرارة الغرفه بحيث لاتزيد عن 16°م. وقد وجد هناك فروق معنويه كبيره فى التعداد اللوغاريتمى للميكروبات فى آخر أيام التخزين (اليوم الرابع) بين العينات المعامله بالتركيزات المختلفه والعينات التى لم تعامل. وقد تم مناقشة الاهميه الصحيه للميكروبات المعزوله والاحتياطات الواجب اتخاذها لتقليل تواجدها والتخلص منها أثناء اعداد السندوتشات لحماية المستهلك.

SUMMARY

One hundred random samples of ready-to-eat sausage, beef burger, shawarma and liver sandwiches (25 of each) were obtained from food restaurants and street-vendors with different sanitation levels in Alexandria City. The samples were examined bacteriologically for aerobic plate count, *Bacillus cereus, Streptococcus faecalis* and *Staphylococcus aureus* count. The obtained results revealed that the mean values of aerobic plate count of sausage, beef burger, shawarma and liver were 3.2± 16x10^4 , 2.3± 1.2x10^4 , 4.2± 2.1x10^4 and 3.6± 1.8 x10^3 cfu /g, respectively. Significant differences were detected among the four different sandwiches. Positive *Bacillus cereus* samples were 100, 92, 84 and 72% with mean values of 2.75± 1.4 x10^4 , 2.4± 1.2x10^2 , 35.2± 17.6 and 3.7± 1.8 x10^3 cfu /g for sausage, beef burger, shawarma and liver, respectively. There was a significant difference between sausage, liver and the other two tested sandwiches. *S. faecalis* was isolated from 88, 84, 88 and 76%, with mean values of 45.7± 5.9 x10^3 , 28± 7x10^2 , 3.1± 1.5 x10^2 and 2± 0.6x10^3 cfu /g for sausage, beef burger, shawarma and liver, respectively. There was a significant difference between sausage, beef burger and the other tested sandwiches. *S. aureus* was isolated from 92, 80, 88 and 80 %, with mean values of 3.25 ± 1.6 x10^3 , 2.8±1.4 x10^2 , 4.1± 2x10^3 and 4.8± 2.4 x10^3 cfu /g, respectively. There was a significant difference between liver, shawarma and the other tested sandwiches. The effect of different concentrations of both freshly crushed Garlic 2, 4 & 6 % and *Nigella sativa* (NS) 1, 3 & 5 % were tested against the isolated *B. cereus, S. faecalis* and *S. aureus* using minced meat stored at room temperature (not more than 16°C). Highly significant differences were recorded between the control and the treated samples. Public health significance and the quality of ready-to-eat meat sandwiches to protect the consumer were given.

Key words: *Street vended foods, meat sandwitches, garlic, Nigella sativa*

INTRODUCTION

Street vended foods are ready-to-eat foods prepared and sold by vendors on street. Street foods provide a source of readily available, nutritional meals, while providing a source of income for the vendors (Bryan *et al.,* 1992; Swanepoel *et al.,* 1995; Ekanem, 1998; Mosupye and Holy, 1999). The vendors congregate mainly in the central business district, nearby new building under construction and at major point of transit, where large numbers of minibus taxi that are headed for different destination. In Egypt, as in other developing countries, street-food-vending has increased markedly because of the increased unemployment rate and limited work opportunities.

Foods are often held for several hours after cooking until sold. This included, for some foods, overnight at ambient temperature, although reheating could alleviate some hazards but this action has not always been done effectively at vending site (Bryan *et al.,* 1988; Bryan *et al.,* 1992). Inadequate reheating is a frequent contributing factor to foodborne outbreaks (Bryan, 1978; Bryan *et al.,* 1988). In contrast to these potential benefits, concerns over the safety and quality of street-vended foods have been raised because the vendors lack an adequate appreciation of basic food safety issues (Moy *et al.,* 1997).

Health risks are associated with initial contamination of raw foods with pathogenic bacteria and subsequent contamination by vendors during preparation from cross-contamination, survival of pathogens during preparation, and microbial proliferation during display (Abou-Zaid *et al.,* 2001; Soliman *et al.,* 2002). The World Health Organization statistics indicated that food-borne diseases may be 300 to 350 times more frequent than the reported cases reveal (Saucier, 1999).

In countries in which street-food vending is prevalent, there is commonly a lack of information on the incidence of food-borne diseases related to street-vended food (Bryan *et al.,* 1988). However, microbiological studies on street-vended foods in America, Asian and African countries have revealed high bacterial counts and high incidences of food-borne bacterial pathogens in food (Bryan *et al.,* 1992; Bryan *et al.,* 1997; Ekanem, 1998). In some cases, street-vended foods have been implicated in outbreaks of food-borne diseases (Dawson and Canet, 1991).

The most common ready-to-eat sandwiches sold by street vendors and fast food restaurants are liver, sausage, brain, spleen, El-hawawshy and shawarma which is a popular meat sandwich in Middle

East Countries (Ayaz *et al.,* 1985).

Bacillus cereus is widely distributed in nature and can be isolated readily from a wide variety of foods in which it may be present normally. Its presence is insignificant, however, unless it is able to grow. Consumption of food containing millions of viable *B. cereus* cells per gram has resulted in outbreaks of food poisoning. Foods incriminated in past outbreaks of *B. cereus* poisoning included vanilla pudding, cooked meat, and vegetable dishes, boiled and fried rice (Geopfert, 1976).

The psychrotrophic strains of *B. cereus,* some capable of producing toxins and evenly grow in foods held at refrigeration temperature have been detected (Buchat *et al.,* 1979; Ahmed *et al.,* 1983; Wood and Waites, 1988; Sutherland and Murdoch, 1994) and those of public health concern. *B. cereus* causes two types of food-poisoning outbreaks, emetic and diarrhea. Several kinds of toxins produced by *B. cereus* strains, such as hemolysin, cytotoxin, emetic toxin and enterotoxins, have been studied (Johnson, 1984; Fagerlund *et al.,* 2007).

Group D streptococci includes, enterococci as *S. faecalis* and *S. faecium* and non enterococci. Enterococci were isolated from shawarma samples collected from fast-food restaurants in Assiut city (Refaie and Moustafa, 1990; Mohamed *et al.,* 2004; Ismail, 2006).

Improper holding of meat products after cooking lead to growth of staphylococci reading without competition with other organisms which have been killed by heat treatment and produced enterotoxin resulting in a staphylococcal food poisoning (Brandly, 1977). The *Staphylococcus aureus* enterotoxins are produced during active growth of the microorganisms in the foods and often during storage. Each enterotoxin is a single poly-peptide chain which resist many proteolytic enzymes and with stands cooking for up to 30 minutes (heat stable) although the vegetative cells would not survive such conditions (Eley, 1992; Baeza *etal.,* 2007).

Nigella sativa (black cumin, black seeds) is important as a cytoprotective herb due to its content of thymoquinone. It is found to increase hepatic GST, as antibacterial, anti-helmintic, antifungal (Toppozada *et al.,* 1965; Agarwal *et al.,* 1979; Hanafy and Hatem, 1991; Hosseinzadeh *et al.,* 2007), immuno-stimulant (El-kadi *et al.,* 1990; Abd-El-Moniem, 1999), anticarcinogenic (Salomi *et al.,* 1992; EL-Gendy *et al.,* 2008), antidiabetic (Abd-El-Aziz *et al.,* 1995), antihistaminic (Chakravarty, 1983), anti-inflammatory, antioxidant and hepatoprotective (Houghton *et al.,* 1995; Youssef and Ashry, 1999). NS crushed seeds supplement stimulate the thyroid gland directly and/or through the pituitary level hence anabolic effect is expected (Khodary *et al.,* 1996). It is a spicy plant and used as flavouring agent for bakery products (Saleh *et al.,* 2002). The antimicrobial activity of *Nigella sativa* seeds were reported by Mahmoud (1993), Sabreen (1996), Abdel-Kader *et al.,* (2001) and AL-Beitawi and EL-Ghousein, (2008).

Garlic (*Allium sativum),* family lilaceae is widely distributed and used in all parts of the world as a spice and herbal remedy for prevention and treatment of variety of diseases due to its active substance Allicin which possesses its characteristic odour (Kamal and Daoud, 2003). Allicin has a broad spectrum antimicrobial effect, it appears to inhibit sulfhydryl enzyme in wide variety of bacteria (Council for Agricultural Science and Technology, 1998; Aydin *et al.*, 2007).

This study was aimed to:

1- Determine the total bacterial count in some popular ready-to-eat sandwiches (sausage, beef burger, shawarma and liver) in Alexandria city.

2- Investigate the occurrence of *B. cereus, S. faecalis* and *S. aureus* in the examined sandwiches.

3- Study the effect of different concentrations of fresh minced garlic and black seed on the viability and survival of the isolated strains of *B. cereus, S. faecalis* and *S. aureus.*

MATERIALS and METHODS

1- Collection of samples:

One hundred ready-to-eat sandwiches were obtained from fast food restaurants and street-vendors with different sanitation levels in Alexandria City. Sandwiches types evaluated were sausage, beef burger, shawarma and liver (25 of each). All samples were obtained aseptically in sterile polyethylene bags. All samples were analyzed immediately after transporting to the laboratory for enumeration of:

a- Aerobic plate count (ICMCF, 1978).

b- *B.cereus* count (Harmon and Goepfert, 1984), the technique of surface spread method was applied using Bacillus cereus selective agar media.

c- *S. faecalis* count (Deibel and Hartman, 1984).

d- *S. aureus* count (ICMSF, 1978) the same technique of surface spread method was applied using Baird-Parker agar media.

2- Preparation of samples:

This was done as recommended by APHA, (1984).

3- Experimental test:

The effect of different concentration of garlic and *N. sativa* on the growth rate of the strains (*B. cereus*, *S. faecalis* and *S. aureus* isolated from ready-to-eat sandwiches) in minced meat stored at room temperature (not more than 16°C) for 4 days was conducted.

Minced meat samples were prepared according to Hefnawy *et al.*, (1993). Meat samples were purchased from local supermarket aseptically in sterile polyethylene bags and divided aseptically into three equal parts, each part was transferred into a sterile glass blender jar. One part was inoculated with strain culture of *B. cereus*, the second part was inoculated with strain culture of *S. faecalis* and the third part was inoculated with strain culture of *S. aureus* as described below.

Test organism:

The organism was grown in 10 ml of nutrient broth at 37°C/ 24h. The culture was decimally diluted and plated to enumerate the organisms present. The culture was diluted to achieve an inoculum level of 10^4/g for *B. cereus* and 10^6/g for *S. faecalis* and *S. aureus*.

Each strain culture was thoroughly mixed with minced meat, then divided into 7parts each part (100g) in sterile beakers. The first one was considered as a control, while the 2nd , 3rd and 4th were mixed with 2, 4 and 6% of garlic (freshly crushed garlic) and 5th , 6th and 7th beakers were mixed with 1, 3 and 5% of *N. sativa* (freshly ground seeds), respectively. All beakers were covered with aluminium foil and stored at room temperature (not more than 16°C) and daily examined for *B. cereus*, *S. faecalis* and *S. aureus*.

RESULTS

Table 1: Statistical analysis of aerobic plate count /g of the examined ready- t0-eat sandwiches (n=25 of each).

Samples	Positive samples		Aerobic plate count/g***			
	NO	%	Min.	Max.	Mean*	S.E.**
Sausage	25	100	3×10^3	4.5×10^5	3.2×10^4B	16×10^4
Beef burger	23	92	2.7×10^3	3.3×10^5	2.3×10^4C	1.2×10^4
Shawarma	24	96	3.3×10^3	5.8×10^5	4.2×10^4A	2.1×10^4
Liver	21	84	2.4×10^2	4.0×10^4	3.6×10^3F	1.8×10^3

***** Means have the same letters are not significant (P<0.05).
****** S.E. = Standard Error.
******* Average of trials

Table (2): Statistical analysis of *B. cereus* count /g of the examined ready-to-eat sandwiches (n= 25 of each).

Samples	Positive samples		*B. cereus* count/g***			
	NO	%	Min.	Max.	Mean*	S.E.**
Sausage	25	100	2.5×10^{3}	3.4×10^{5}	2.75×10^{4}B	1.4×10^{4}
Beef burger	23	92	2×10	3.1×10^{3}	2.4×10^{2} J	1.2×10^{2}
Shawarma	21	84	2.7×10	4.0×10^{2}	35.2 K	17.6
Liver	18	72	2.8×10	4.2×10^{4}	3.7×10^{3} F	1.8×10^{3}

*

Means have the same letters are not significant (P<0.05).
** S.E. = Standard Error.
*** Average of trials

Table 3: Statistical analysis of *S. faecalis* count /g of the examined ready-to-eat sandwiches (n= 25 of each).

Samples	Positive samples		*S. faecalis* count/g***			
	NO	%	Min.	Max.	Mean*	S.E.**
Sausage	22	88	12×10^{3}	12×10^{4}	45.7×10^{3}A	5.9×10^{3}
Beef burger	21	84	10^{2}	11.4×10^{3}	28×10^{2} G	7×10^{2}
Shawarma	22	88	2.7×10	3.5×10^{3}	3.1×10^{2} I	1.5×10^{2}
Liver	19	76	1×10	9×10^{3}	2×10^{3} H	0.6×10^{3}

* Means have the same letters are not significant (P<0.05).
** S.E. = Standard Error.
*** Average of trials.

Table 4: Statistical analysis of *S. aureus* count /g of the examined ready-to-eat sandwiches (n= 25 of each).

Samples	Positive samples		*S. aureus* count /g***			
	NO	%	Min.	Max.	Mean*	S.E.**
Sausage	23	92	2.7×10^{2}	4.15×10^{4}	3.25×10^{3}G	1.6×10^{3}
Beef burger	20	80	2.3×10	3.95×10^{3}	2.8×10^{2} I	1.4×10^{2}
Shawarma	22	88	3.4×10^{2}	5.2×10^{4}	4.1×10^{3} E	2×10^{3}
Liver	20	80	3.7×10^{2}	6×10^{4}	4.8×10^{3}D	2.4×10^{3}

* Means have the same letters are not significant (P<0.05).
** S.E. = Standard Error.
*** Average of trials.

Storage	Log_{10} cfu /g

period /day	*B. cereus* + Garlic				*B. cereus* + *N. sativa*		
	control	2%	4%	6%	1%	3%	5%
0	4.3 E	4.3 E	4.3 E	4.3 E	4.3 E	4.3 E	4.3 E
1	5.1 D	3.8 F	3.5 G	3.1 H	3.1 H	1.3 L	2.5 J
2	5.6 C	3.5 G	1.1 M	1.4 L	2.8 I	1.1 M	2.3 K
3	6.3 B	2.8 I	0.0 N	0.0 N	0.0 N	0.0 N	0.0 N
4	6.5 A	0.0 N	0.0 N	0.0 N	0.0 N	0.0 N	0.0 N

Table 5: Effect different concentrations of Garlic and *Nigella sativa* on *B. cereus*.

Log_{10} cfu /g have the same letters are not significant (P<0.05).

Table 6: Effect different concentrations of Garlic and *Nigella sativa* on *S. faecalis*.

Storage period /day	Log_{10} cfu /g						
	S. faecalis + Garlic				*S. faecalis* + *N. sativa*		
	control	2%	4%	6%	1%	3%	5%
0	6.5 F	6.5 F	6.5 F	6.5 F	6.5 F	6.5 F	6.5 F
1	6.7 D	6.8 C	6.5 F	6.5 F	6.5 F	6.5 F	6.2 G
2	6.8 C	6.7 D	6.5 E	5.8 I	6.6 E	6.0 H	5.8 I
3	7.7 B	5.5 J	5.5 J	5.8 I	6.2 G	5.8 I	5.5 J
4	8.2 A	5.3 K	5 L	4.6 N	6.0 H	5.3 K	4.9 M

Log_{10} cfu /g have the same letters are not significant (P<0.05).

Table 7: Effect different concentrations of Garlic and *Nigella sativa*on *S. aureus*.

Storage period /day	Log_{10} cfu /g						
	S. aureus + Garlic				*S. aureus* + *N. sativa*		
	control	2%	4%	6%	1%	3%	5%
0	6.1 F	6.1 F	6.1 F	6.1 F	6.1 F	6.1 F	6.1 F
1	6.3 D	6.4 C	6.1 F	6.1 F	6.1 F	6.1 F	5.8 G
2	6.4 C	6.3 D	6.1 F	5.4 I	6.2E	5.6 H	5.4 I
3	7.3 B	5.1 J	5.1 J	5.4 I	5.8 G	5.4 I	5.1 J
4	7.6 A	4.9 K	4.5 M	4.3 O	5.6 H	4.8 L	4.4 N

Log_{10} cfu /g have the same letters are not significant (P<0.05)

DISCUSSION

The results recorded in Table (1), reveal that the aerobic plate count of the examined ready-to-eat sausage sandwiches ranged from $3x10^3$ to 4.5×10^5 with a mean value of $3.2x10^4 \pm 16 \times 10^4$ cfu/g.Our results are lower than those of Abou-Zaid *et al.,* (2001) who recorded $3x\ 10^8$ cfu/g and higher than those of Soliman *et al.,* (2002) who recorded $3.2x10^2$ cfu/g.

As regarded to beef burger ready-to-eat sandwiches, the total aerobic count varied from $2.7x10^3$ to $3.3x10^5$ with a mean value of $2.3x10^4 \pm 1.2x10^4$ cfu/g. Nearly similar results were obtained by Mohamed (1997), while lower results were obtained by Mohamed (1985), Gill *et al.,* (1996) and Soliman *et al.,* (2002).

Although the aerobic plate count of any food article is not a sure indicative of its safety for consumption, yet it is of supreme importance in judging the hygienic conditions under which it has been produced, handled and stored (Levine, 1961).

Concerning shawarma sandwiches, the APC varied from $3.3x10^3$ to $5.8x10^5$ with a mean value of $4.2x10^4 \pm 2.1x10^4$ cfu/g. These results are lower than those reported by Ayaz *et al.,* (1985), Refaie and Moustafa, (1990) and Ebraheem, (2001) who recorded a mean APC of 10^2 to $3.0x10^6$, $24.6x10^7$ and $67.88 \pm 31.7x10^6$ cfu/g ready-to-eat shawarma sandwiches, respectively. On the other hand, Morshedy *et al.,* (1986) and Mohamed et al., (2004) recorded higher results ($38x10^2$ and $29 \pm 4.9x10^3$ cfu/g, respectively).

The results recorded in Table (1), reveal that the aerobic plate count of the examined ready-to-eat liver sandwiches ranged from $2.4x10^2$ to $4.0x10^4$ with a mean value of $3.6x10^3 \pm 1.8x10^3$ cfu/g. Nearly similar results were obtained by Mohamed *et al.,* (2004) ($27 \pm 18x10^3$ cfu/g). Higher counts were recorded by Hegazy (1999), Ebraheem (2001) and Abou-Zaid *et al.,* (2001) were the last one recorded a mean value of $2x10^7$ cfu/g.

Table (1), also showed that there were significant differences ($P > 0.05$) among the four different sandwiches studied. The highest contaminated sandwiches were shawarma and sausage. Bacterial counts in the sandwiches analyzed in the present study were found to be lower than those reported by Refaie and Moustafa, (1990). However, the APC recorded by Mouspye and Holy, (1999) in meat samples collected from street-vendors in Johannesburg, South Africa were lower than ours (3.1± 0.9 log cfu/g). However, in other countries as Pakistan, Zambia and Nigeria higher results were recorded for street-vended cooked meals

(Bryan *et al.,* 1992; Ekanem 1998; Mouspye and Holy, (1999).

According to the microbiological standard for hot meals recorded by Lufthansa and Swiss Air Service, (1986) it is clearly evident that the means of APC for sausage, beef burger, shawarma and liver were less than 10^5 cfu/g which is considered within the permissible limits.

Table (2), shows that the minimum, maximum and means of *B.cereus* counts in sausage ready-to-eat sandwiches were $2.5x10^3$, $3.4x10^5$ and $2.75x10^4 \pm 1.4x10^4$ cfu/g, respectively. *B.cereus* was isolated from 100% of sausage sandwiches. Our results were higher than those of Abou-Zaid *et al.,* (2001) who recorded 40% positive samples with a mean value of $7x10^4$cfu/g and Soliman *et al.,* (2002) who reported $3.1x10^2$ cfu/g of ready-to-eat sausage sandwiches.

The recorded data in Table (2), showed that 92% of the examined ready-to-eat beef burger sandwiches were positive for *B.cereus* with count ranged from 2 x10 to $3.1x10^3$and a mean value of $2.4x10^2 \pm 1.2x10^2$ cfu/g. Nearly similar results ($2.51x10^2$ cfu/g) were obtained by Soliman *et al.,* (2002).

B.cereus was isolated from 84% of the examined shawarma sandwiches with count ranged from 2.7x10 to $4.0x10^2$ with a mean value of 35.2± 17.6 cfu/g. However, higher incidence (88%) and count ($2x10^3 \pm 4x10^2$ and $28 \pm 7x10^2$ cfu/g) were reported by Nassar *et al.,* (2003) and Mohamed *et al.,* (2004).

In Table (2), the minimum, maximum and the means of *B.cereus* counts in liver ready-to-eat sandwiches were 2.8x10 to $4.2x10^4$ and $3.7x10^3 \pm 1.8x10^3$ cfu/g, respectively. Nearly similar results were obtained by Abou-Zaid *et al.,* (2001) (10^3cfu/g) and Mohamed *et al.,* (2004) ($2 \pm 0.6x10^3$ cfu/g). Hegazy, (1999) recorded little higher count ($10^4 \pm 6x10^3$ cfu/g), while Ebraheem, (2001) recorded 100% positive samples with a mean value of 99.6± 7.9 cfu/g.

The results obtained in this study showed significant differences ($P>0.05$) the in the mean values of *B.cereus* count among four different sandwiches studied. The highest contaminated sandwiches were sausage and liver (Table 2).

Members of the genus Bacillus are ubiquitous soil microorganisms and are generally considered harmless contaminants. However, a few species are known as toxin producers, including the food borne pathogen, *B.cereus*. It is observed from the achieved results that *B.cereus* contaminated very high percentage of the examined ready-to-eat sandwiches and this may due to the fact that *B.cereus* is widely distributed in nature. Kramer and Gilbert, (1989) stated that *B.cereus* population between 10^5 and 10^7cells/g of food is required to produce intoxication. Accordingly, the level of contamination with *B.cereus* appeared to be not significant to public health.

As recorded in Table (3), *Streptococcus faecalis* were existed in the examined ready-to-eat sausage sandwiches in numbers varied from $12x10^3$ to $12x10^4$ with a mean value of $45.7x10^3 \pm 5.9x10^3$ cfu/g, the count of *S. faecalis* in beef burger ranged from 10^2 to $11.4x10^3$ with a mean value of $28x10^2 \pm 7x10^2$ cfu/g, In shawarma from 2.7x10 to $3.5x10^3$ with a mean value of $3.1x10^2 \pm 1.5x10^2$ cfu/g and from 10 to $9x10^3$ with a mean value of $2x10^3 \pm 0.6x10^3$ cfu/g in the tested ready-to-eat liver sandwiches, respectively.

A higher results in both count and percentage of positive samples were recorded by Elwi, (1988) who reported $5x10^5$ cfu/g and nearly similar results were obtained by Mohamed et al., (2004) with a mean value of $26.7 \pm 6x10^2$ cfu/g for cooked liver. Morshedy *et al.*, (1986) and Ebraheem, (2001) recorded nearly similar count ($6x10^2$ cfu/g) in shawarma but, Mohamed *et al.*, (2004) reported a mean value of $28 \pm 7x10^2$ cfu/g which seemed to be higher.

Significant differences in the means of *S. faecalis* count between sausage, and beef burger and both liver and shawarma sandwiches were observed. The highest contaminated sandwiches were sausage, and beef burger (Table 3)

Mohs (1972) reported a level of $<10^3$ /g of *S. faecalis* as an acceptable bacteriological quality in cooked foods. The examined beef burger and shawarma sandwiches comply with this limit. The presence of *S. faecalis* in the examined ready-to-eat sandwiches may be due to post-processing contamination or heat resistant character of the organism.

Table (4),shows that the minimum, maximum and the means of *Staphylococcus aureus* counts in sausage ready-to-eat sandwiches were $2.7x10^2$, $4.15x10^4$ and $3.25x10^3 \pm 1.6x10^3$ cfu/g, respectively. Our results were higher than those of Abou-Zaid *et al.*, (2001) who reported $4x10^2$ cfu/g and Soliman *et al.*, (2002) who recorded a mean value of $3.25x10^2$ cfu/g of ready-to-eat sausage sandwiches. As for ready-to-eat beef burger sandwiches 80% were positive for *S. aureus* where the count ranged from 2.3x10 to $3.95x10^3$ with a mean value of $2.8x10^2 \pm 1.4x10^2$ cfu/g. Nearly similar findings $2.1x10^2$ cfu/g were obtained by Soliman *et al.*, (2002), while higher results were reported by Mohamed, (1997).

Hoshyar *et al.*, (1984) and Mohamed, (1985) obtained nearly similar results as regard *S. aureus*. Growth of *S. aureus* can occur either before or during processing of meat so that the meat and its products have been implicated in several food poisoning outbreaks (Niskanen, 1977). While *S. aureus* in food indicates its contamination from the skin, mouth or nose of workers handling and inadequately cleaned equipments (Thatcher and Clark, 1978).

It is worth to mention that 88% of the examined ready-to-eat shawarma sandwiches were positive for *S. aureus* with count ranged from $3.4x10^2$ to $5.2x10^4$ and a mean value of $4.1x10^3 \pm 2x10^3$ cfu/g. Soliman *et al.,* (2001) recorded low results $2x10^2$ cfu/g (Table 4). However, ready-to-eat liver sandwiches (80%) contained *S. aureus* ranged from $3.7x10^2$ to $6x10^4$ and a mean value of $4.8x10^3 \pm 2.4x10^3$ cfu/g. These results were lower than those of Soliman *et al.,* (2001) who reported 10^2 cfu/g. Significant difference was observed in the means of *S. aureus* count among beef burger and sausage, shawarma and liver.The highest contaminated sandwiches were liver and shawarma (Table4).

Tables (5,6&7), show the effect of different concentrations of Garlic and *Nigella sativa* on *B. cereus, S. faecalis* and *S. aureus* isolated from ready-to-eat sandwiches, in minced meat stored at room temperature (not more than 16°C). The effect of 2, 4 and 6% Garlic decreased the numbers of *B. cereus* by 4.3 orders of magnitude in the 4^{th}, 3^{rd} and 3^{rd} days of storage comparable to zero time and 6.3 and 6.5 orders of magnitude in the 3^{rd} and 4^{th} days of storage comparable to control, respectively. While these concentrations decreased the numbers of *S. faecalis* 1.2, 1.5 and 1.9 orders of magnitude in the 4^{th} day of storage comparable to zero time and 2.9, 3.2 and 3.6 orders of magnitude comparable to control in the last day of storage, respectively (Table 6). On the other hand, these concentrations decreased the numbers of *S. aureus* 1.2, 1.6 and 1.8 orders of magnitude in the 4^{th} day of storage comparable to zero time 2.7, 3.1 and 3.3 orders of magnitude comparable to control in the last day of storage, respectively (Table 7).

No significant differences were observed between the different concentrations of *N. sativa* on the 3^{rd} day of storage on *B. cereus* (Table 5) and of the numbers of organisms in the last day of storage decreased by 4.3 orders of magnitude comparable to zero time and by 6.5 orders of magnitude comparable to the control regardless to the concentration of *N. sativa* used with *B. cereus*. Table (6), show that the numbers of *S. faecalis* decreased by 0.5, 1.2 and 1.6 log cycles at concentrations of 1, 3 and 5% of *N. sativa* comparable to zero time and by 2.2, 2.9 and 3.3 log cycles as compared with control in the last day of storage, respectively. Finally, Table (7) show that the numbers of *S. aureus* decreased by 0.5, 1.3 and 1.7 log cycles at concentrations of 1, 3 and 5% of *N. sativa* comparable to zero time and by 2, 2.8 and 3.2 log cycles as compared with control in the last day of storage, respectively. Our results are in agreement with those obtained by Allatif and Ibraheem, (1996). The antibacterial effect of both Garlic and *N. sativa* was recorded in many other studies (Mahmoud, 1993; Council for Agricultural Science and Technology, 1998; Abdel-Kader *et al.,* 2001; Mohamed *et al.,* 2004).

In these studies, high bacterial counts and high incidence of food-borne pathogens have been attributed to a process by which food are held for long period of time at temperature of 46°C. And also, it was reported that vendors cooked their foods on temperature lower than 95°C, but they then held the food at ambient temperature for more than 6 h. and sometimes even held it overnight without reheating (Bryan *et al.*, 1997). They also added that the presence of contaminating bacteria in the food was attributed to cross-contamination from environmental sources and to handling by vendors during holding. High percentage of these samples contained high number of *B. cereus*, *S. faecalis* and *S. aureus*, it is indicated improper sanitation and neglected hygienic measures during preparation causing sandwiches of inferior quality, unfit for consumption, index of fecal contamination and possibility results of presence of enteric pathogens.

From the previous results we concluded that Garlic and *N. sativa* play a significant role in prevention of bacterial growth, so must be added to sandwiches to improve the quality of ready-to-eat sandwiches. Services the ten golden rules drawn by (WHO, 1989). Educational programs and training courses should be recommended to the meat handlers and workers. Periodical cleaning of clothes, utensils, hands especially after visiting toilet and protect foods from (dust, insects, avoid keeping of warm below 60°C and food may only be stored for a limited time). Consumers should be informed about the risk of foods contamination during the months of year (Gongal, 1998).

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Samples	Positive	*B. cereus* count/g*******

	samples		Min.	Max.	Mean*	S.E.**
	NO	%				
Sausage	25	100	2.5×10^{3}	3.4×10^{5}	2.75×10^{4}B	1.4×10^{4}
Beef burger	23	92	2×10	3.1×10^{3}	2.4×10^{2} J	1.2×10^{2}
Shawarma	21	84	2.7×10	4.0×10^{2}	35.2 K	17.6
Liver	18	72	2.8×10	4.2×10^{4}	3.7×10^{3} F	1.8×10^{3}