READY-TO-EAT MEAT AND POULTRY PIES AS A SOURCE OF POTENTIAL PATHOGENS IN ASSUIT CITY

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ABSTRACT

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Atotal of 80 samples of pies, 40 each of meat and poultry pies were collected from different restaurants of Assiut city and analyzed bacteriologically (count of aerobic bacteria, coliform, faecal coliform Staph aureus, E.coli and isolation of Staph aureus, Clostridium perfringens, Escherichia coli and Listeria monocytogenes). The mean values of aerobic plate count and Staph. aureus counts were 8×10^4 and 5X10cfu/g of examined meat pies while that of poultry pies were 8.3 x $10^5$and 6 x 10^2 cfu/g respectively. A signifhcant difference in such counts was observed between the two types of pies examined. Regarding the MPN of coliform and faecal coliform, the mean values were 1.1X10 and 0.5X10 for meat pies whereas the corresponding values for poultry pies were 0.7 X10 and 0.5 X10 respectively. However, Staph. aureus, Clostridium perfringens, Escherichia coli and Listeria monocytogenes were isolated from 82.5,17.5,7.5 and 15% of the examined meat pies while poultry pies contained such organisms in 77.5,12.5,12.5 and 22.5% of the examined samples. The public health importance of recovered microorganisms as well as some recommended measures for improving the quality of such products were discussed.

Key words: *Meat pies, poultry pies, potential pathogens.*

فطائر اللحوم والدجاج المعدة للأكل كمصدر محتمل لمسببات الأمراض في مدينة أسيوط

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لقد تم جمع ٨٠ عينة من الفطائر بواقع ٤٠عينة من فطائر اللحم و٤٠ اخرى من فطائر الدجاج من مطاعم مختلفة بمحافظة أسيوط واجرى الفحص البكتريولوجى لها حيث تم تحديد العدد الكلى لكل من: الميكروبات الهوائية، ميكروبات القولون، القولون البرازية، ميكروب المكور العنقود الذهبى والايشريشية القولونية. وقد تم عزل كل من الميكروب المكورالعنقودي الذهبي والكلوستريديم بيرفرنجنز والايشريشية القولونية والليستيريا مونوسيتوجين حيث وجدت ان المتوسطات للميكروبات الهوائية والميكروب المكورالعنقود الذهبى٨ ×١٠^{٤}، ٥X١٠/جرام وذلك فى فطائر اللحوم بينما كانت هذه المتوسطات فى فطائر الدجاج ٨.٣× ١٠^{٥} و٦×١٠^{٢}/جرام بالترتيب. ولقد وجد اختلاف معنوى بين هذه المتوسطات فى نوعى الفطائر التى فحصت. اما بالنسبة للعدد الكلى المحتمل للميكروب القولونى والميكروب القولونى البرازى كانت المتوسطات لها١.١×١٠و٥.٠×١٠ بالنسبة لفطائر اللحوم بينما كانت هذه المتوسطات فى فطائر الدجاج ٧.٠×١٠و٥.٠×١٠بالترتيب. وتم عزل الميكروب المكورالعنقود الذهبى والكلوستريديم بيرفرنجنز والايشريشية القولونية والليستيريا مونوسيتوجين من فطائر اللحوم بالنسب الاتية: ٨٢.٥، ١٧.٥ ، ٧.٥، ١٥% بالترتيب بينما عزلت هذه الميكروبات من فطائر الدجاج بالنسب الاتية: ٧٧.٥، ١٢.٥ ، ١٢.٥ ، ٢٢.٥%بالترتيب. وقد تمت مناقشة الأهمية الصحية لهذه الميكروبات ومدى خطورتها على الصحة العامة كذلك الطرق المقترحة للحد من هذه الخطورة.

INTRODUCTION

Ready-to-eat foods could be raw or cooked, hot or chilled and can be consumed without further heat treatment (Tsang, 2002).

Examples of such foods included meat pies and poultry pies. This situation however has resulted in more ready to-eat-foods taken outside home, this food vendor services become on the increase and responsibility for good manufacturing practices of food such as good. The sanitary measures and proper food handling must be improved, because venders can transfer diseases between families through ready-to-eat foods (Musa and Akande, 2002).

Mishandling in food service establishment can contribute significant outbreaks of foodborne disease (Frazier and Westhoff, 2001), also these outbreaks are caused by foods that are contaminated intrinsically (Torok *et al.,* 1997). Because of modern processing methods, handling and distribution, it takes longer for food to reach the table, and it is more likely to be contaminated with microorganisms. (Marriot, 1997).

Staph. aureus, Clost. perfingens and *Escherichia coli* are bacteria that cause food poisoning. Food infection is the second type of foodborne illness. It is caused by eating food that contain certain types of live bacteria which are present in the food. Once the food is consumed, the bacterial cells themselves continue to grow and illness can result, *Listeria* is a good example of foodborne infection. Each year, thousands of individuals suffer from the discomfort and pain resulting from foodborne illness. True food poisoning or food intoxication caused by eating food that contains a toxin or poison due to bacterial growth in food (Estes *et al.,* 2003).

It is extremely difficult to eliminate contamination of food completely, but it is easier to control further multiplication of pathogens and stop the forms that produce toxins (Bryan *et al.,* 1991). The bacteria which produced and excreted the toxic waste products into the food may be killed, but the toxin they produced causes the illness or digestive upset to occur (Estes *et al.,* 2003).

Prevention of foodborne infections is based on: pathogen-free food production, hazard control in food processing, surveillance for foodborne illness, and safe food handling by consumers and food-service workers (Doyle, 1993). The practices developed by professionals to ensure the safety of food production and processing are based primarily on knowledge of microbiology (Altekruse *et al.,* 1996).

Since the rules of how to avoid food poisoning are known, the problem in mass catering is enforcement which must include not only proper equipment and installations but also training of personnel (Teuber, 1992).

As there is scarce of information about the bacterial status of pies in Assiut City therefore the aim of this study was focused to assess the bacteriological quality of meat and poultry pies in Assiut governorrate.

MATERIALS and METHODS

To ascertain the levels and types of bacteria present in meat and poultry pies, 40 samples of each ready-to-eat products were obtained from randomly selected restaurants of Assiut City and examined on the day they were collected for:

1- Total colony count (APC): APHA (1992).

2- Coliform count, Faecal coliform count, *E.coli* count (MPN/g): AOAC (1990).

3- *Staph. aureus* count: APHA (1992).

4- Isolation of *Staph aureus*: Feingold and Martin (1982).

5- Isolation of *Listeria monocytogen:* Oxoid Manual (1990).

6- Isolation of *Clostridium perfringens*: Beernes *et al.* (1980).

7-Isolation of *E.coli*: AOAC (1990).

All isolates were identified morphologically using staining reaction (APHA, 1992) and motility test (Baron *et al.,* 1994), as well as, biochemically using catalase, coagulase and triple sugar iron (TSI) agar test (Baron *et al.,*

1994), citrate utilization, indole production, methyl red, urease and voges-Proskauer tests (Koneman *et al.,* 1992), nitrate reduction test (Cowan and Steel, 1974), sugar fermentation reaction (APHA, 1992), Christine-Alkine-Munch- Peterson (CAMP) test (Herrera, 2001). For further confirmation of *L.monocytogenes* the isolates were inoculated into 10% aqueous stock solution of Mannitol, L.Rhammose and D.xylose (Collee and Miles, 1989).

RESUTS

Table1: Statistical values of aerobic plate count / gram of the examined meat and poultry pies.

	No of exam samples	No.of + ve samples	Minimum	Maximum	Mean	S.E	P. value
Meat pies	40	40	9×10^3	2.9×10^5	8 x 104	$\pm 1 \times 10^4$	< 0.01*
Poultry pies	40	40	2.6×10^4	6.9×10^6	8.3×10^5	$\pm 4.3 \times 10^5$	

Table 2: Statistical values of *Sstaph aureus* count / gram of the examined meat and poultry pies.

	No of exam samples	No.of+ ve samples	Minimum	Maximum	Mean	S.E	P. value
Meat pies	40	31	1 x 10	2×10^2	5 x 10	1.4 x 10	< 0.01*
Poultry pies	40	30	4 x 10	3×10^3	6×10^2	5×10^2	

Table 3: Statistical values of coliform count (MPN) / gram of the examined meat and poultry pies.

	No of exam samples	No.of+ ve samples	Minimum	Maximum	Mean	S.E	P. value
Meat pies	40	40	3.6	4.3 x 10	1.1 x 10	± 0.1 x 10	N.S
Poultry pies	40	38	3.6	23	0.7 x 10	± 0.1 x 10	

Table 4: Statistical values of faecal coliform count (MPN)/gram of the examined meat and poultry pies.

	No of exam samples	No.of+ ve samples	Minimum	Maximum	Mean	S.E	P. value
Meat pies	40	36	3.6	1.1 x 10	0.5 x 10	± 0.04 x 10	N.S
Poultry pies	40	35	3.6	1.5 x 10	0.5 x 10	± 0.08 x 10	

N.S: non significant
* : Significant

Table 5: Frequency distribution of meat and poultry pies based on their E.coli count (n=40 of each).

Interval	Meat pies		Poultry pies	
	No.	%	No.	%
<3	38	95	37	92.5
3-<6	1	2.5	3	7.5
6-<9	1	2.5	-	-

Table 6: Incidence of the isolated organisms from the examined meat and poultry pies.

Organisms	Meat pies			Poultry pies		
	No. of exam. samples	No. of +ve samples	%	No. of exam. samples	No. of +ve samples	%
Staph aureus	40	33	82.5	40	31	77.5
C.perfrengenes	40	7	17.5	40	5	12.5
E.coli	40	3	7.5	40	5	12.5
L.monocytogenes	40	6	15	40	9	22.5

DISCUSSION

Regulatory agencies and industrial quality assurance regularly examine foods or ingredients for microorganisms or their metabolic productsthat may indicate: (1) The possible presence of a pathogen or harmful toxins, (2) The possibility that faulty practices occurred during production, processing, storage and distribution, and for (3) The suitability of a food or ingredient for a desired purpose (NAS, 1985).

The standard plate count is one of the most common tests applied to indicate the microbiological quality of food. High count may indicate the product may have been prepared unhygienically or stored inappropriately (ICMSF, 2001).

The results recorded in Table 1 reveal that all the examined samples (100%) contained arobic bacteria in numbers varied from 9 x 10^3 to 2.9 x 10^5 with a mean value of 8 ± 1 x 10^4 CFU/g, in meat pies while in poultry pies the numbers varied from 2.6 x 10^4 to 6.9 x 10^6 with a mean value of 8.3 ± 4.3 x 10^5 CFU/g. There was a comparable difference ($P < 0.01$) between the mean total microbial counts between meat pies and poultry pies.

The high microbial counts may be due to meat offers a rich nutrient media for microbial growth (Phillips, 2003). Also this is an indication of recontamination in food handling hygiene techniques starting from the processing of the raw material to the finished product (Ikeme, 1990; Ojeibun, 1994). Higher counts of aerobic bacteria in meat pies were also enumerated by Alexander and Tittiger (1971), and a lower figure 3 x 10^3 – 5 x 10^3 CFU/g was reported by Yah clarence *et al.* (2009).

Table 2 showed the statistical values of *Staph aureus* (CUF/g) of the examined meat and poultry pies, the count ranged between 1 x $10^1$and 2 x 10^2 with a mean value of 5 ± 1.4 x 10^1 CFU/ g meat pies and ranged between 4 x 10^1 and 3 x 10^3 with a mean value of 6 ± 5 x 10^2 CFU/g in poultry pie, that a significance difference was apparent between them ($P < 0.01$). Higher counts of *Staph aureus* in meat pies were recorded by El-Gohary (1994) and Yah Clarence *et al.* (2009).

The significance difference ($P < 0.01$) which showed in both of total microbial counts and *Staph aureus* counts between meat pies and poultry pies could be attributed to the ingredients used and the processing difference.

Results of coliform count (MPN/g) indicated in Table 3 verify that the minimum value of coliform in meat pies was 3.6, the maximum value was 4.3 x 10^1 and the mean value was 1.1 x 10^1 ± 0.1 x 10^1, while in poultry pies the minimum, maximum and mean values were 3.6,23 and 0.7 x 10^1 ± 0.1 x 10^1, respectively.

According to the data summarized in Table 4, the faecal coliform count (MPN/g) varied from 3.6 to 1.1 x 10^1 with a mean value of 0.5 x 10^1 ± 0.04 x 10^1 in meat pies and this count varied from 3.6 to 1.5 x 10^1 with a mean value of 0.5 x 10^1 ± 0.08 x 10^1 in poultry pies.

Regarding meat pies, two samples had *E.coli* where the MPN for each lied between 3-<6 and 6-<9/g whereas poultry pies revealed the presence of *E.coli* in 3 samples only where the MPN lied between 3-<6/g as recorded in Table 5.

There was no significant difference between meat pies and poultry pies in coliform and faecal coliform counts as recorded in Tables 3 and 4.

Results given in Table 6 revealed that 82.5% and 775% of *Staph aureus* were isolated from meat and poultry pies respectively, this percent was higher than that obtained by Alexander and Tittiger (1971) (22.7%) in meat pies. *Staphylococcus* is a true food intoxication organism. It produces a heat stable toxin when allowed to grow for several hours in foods such as chicken pies. This bacterial growth may not cause any off color, odor, or textural or flavor changes, but the toxin will be secreted into the food. This organism grows best at body temperature (36.6 ^oC), but it can grow over the much wider range of 10 ^oC to 46.1^{o}c. the best prevention of *staph* food poisoning is to

properly store food and reduce the temperature below 40 degree F within four hours after preparation of serving. In order for *Staph* to grow and produce toxin, it must have sufficient time, approximately two to four hours. Therefore, it is important to cool or heat foods through the danger zone of 4.4^{o}C to 60^{o}C as rapidly as possible.

C.perfringens was found in 7 samples of meat pies (17.5%) and in 5 samples of poultry pies (12.5%) (Table 6). Alexander and Tittiger (1971) reported that clostridia were found in low numbers in some samples of meat pies.

C.perfriongens can grow over a wide range of temperature, but grows very slowly at low temperatures, these bacterial spores will germinate and grow best at temperatures between 37.7◦ C and 47.2◦C.

Many foods such as meat and poultry may carry the organism, but the mere presence of *C.perfringens* in food is not enough to cause illness. Millions of growing cells are needed. The prevention of growth of this organism is best accomplished by following the standard food service practices of rapidly chilling prepared foods in shallow containers and keeping cold food cold and hot food hot, also reduce the level of contamination by keeping all work areas clean and sanitary (Estes *et al.,* 2003).

Results given in Table 6 revealed that 7.5% of *E-coli* were isolated from 3 out of 40 samples of meat pies and 12.5% were isolated from 5 out of 40 sampels of poultry pies. Alexander and Tittiger (1971) reported that the incidence of samples (Meat pies) containing coliform was high (81.6%), while Yah Clarene *et al.* (2009) cited the count of *E.coli* in meat pies as 2 x 10^3 CFU/g.

According to Edema *et al.* (2001), Okonko *et al.* (2008 a, b) the presence of *E.coli* in food is an indication of faecal contamination of the water sources that were utilized in the processing of these food products. Also Edema *et al.* (2005) reported that biological contaminants of bacterial origin present as major cause of foodborne disease given rise to acute to chronic illnesses such as *E.coli* gastroenteritis, brucellosis and campylobacteriosis.

Listeria monocytogenes was detected in our study in 15% of meat pies and in 22.5% of poultry pies (Table 6).

This organism is a food infection bacteria gaining in public awareness as a safety problem in food products. The general growth conditions required are oxygen, temperatures ranging from 2.7 C° to 40 C° and a pH range of 5.6 to 9.8, since *Listeria* can grow at refrigerated temperatures. The organism is generally destroyed by heat treatment, 76.6 °C for 15 seconds. Proper personal hygiene, good sanitation, proper cooking and preventing cross contamination of raw and cooked food are the best control measures known to date (Estes *et al.,* 2003).

Thus to safeguard against the risks of detected microorganisms, there is need to educate and advocate for good manufacturing practices among food processors and food vendors.

Betty and Richard 1994, said that food poisoning / illnesses are entirely preventable by practicing good sanitation and food handling techniques.

In conclusion, a mong the requirement of any food to be of good sanitary quality, it must be free from hazardous microorganisms, or these present should be at a safe level. Therefore, standards for composition and bacterial content are now adopted by nearly all countries. so that public may be assured of a safe healthful product.

The information given by the achieved results proved that most of the examined meat and poultry pies contained valiable numbers and types of microorganisms which may be responsible for inferior quality of the product and increase the risk of public health. Therefore, food safety standards should be applied and Hazard Analysis Critical Point (HACCP) should be applied throughout processing and distribution of the product.

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