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Bacterial Contamination of Fast Foods

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Abstract

A grand total of 120 random samples of ready to eat sandwiches of meat products represented by beef kofta, beef burger, sausage and liver (30 of each) were collected to be evaluated bacteriologically. Liver samples showed a significant increase of Aerobic Plate count, total Staphylococcus and staph. aureus count (cfu/g) than kofta and burger samples, while sausage samples showed an increase than burger samples. In addition, liver samples showed a significant increase of coliform count (cfu/g) than burger samples. Actually *E. coli* was isolated from beef burger, kofta and sausage and liver samples have the same incidence of occurrence 16.7% and 33.3%, respectively. The incidence of Staph. aureus in beef burger, kofta, sausage and liver sandwiches were 14.3%, 19%, 28.6% and 38.1%, respectively. In addition, the incidence of Staph. aureus producing enterotoxins sea, sab, sec and sed were 33.3%, 16.6%, 16.6% and 8.3%, respectively. Citrobacter diversus, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Klebriella ozaenae, Klebriella pneumonia, Proteus mirabilis, Proteus vulgaris and Providencia rettgeri were isolated with varying percentages. As conclusion, ready to eat meat products were contaminated with different types of microorganisms. Liver samples showed the highest contamination level.

Key words: Bacterial contamination, Fast foods, PCR, RTE.

1. Introduction

Ready–to–eat (RTE) foods reflect consumer demand for convenient foods, consumer are looking for RTE foods that are fresh, healthy, safe, additive free and nutritious [15]. In Egypt, the most RTE sandwiches sold in street vendors and fast food restaurants are beef kofta, beef burger, sausage and liver. Meat products can be easily contaminated with microorganisms leading to loss of quality and potential public health problems. [43]. Also, addition of certain spices during manufacture may lead to marked increase in the bacterial population [39]. The most important bacteria causing food poisoning include *E. coli* and Staphylococcus aureus [25]. Presence of *E. coli* in RTE foods indicates that the food has been prepared under poor hygienic conditions [27]. E.coli is considered as an indicator of fecal contamination [40]. While, Staphylococcal food poisoning is the result of preformed enterotoxins that are produced by certain strains of Staph. aureus [36]. The presence of Staph.aureus in heat treated food is a pointer to largely poor personal hygiene, improper storage facilities, and unhygienic environment [4]. Other studies have also demonstrated that, Enterobacteriaceae group has an epidemiological interest as some of its members are pathogenic and may result in serious infections and food poisoning [30].

Therefore, the present study was carried out to determine APC, coliform, Staphylococcal and Staph. aureus counts on samples of cooked burger, kofta, sausage and liver. Isolation, identification of Enterobacteriaceae, *E. coli*, Staph. aureus and detection of staph. aureus enterotoxins by using PCR, were also determined.

2. Material and methods

2.1 Samples collection

A grand total of 120 random samples of ready to eat meat products sandwiches represented by beef koft ،beef burger، sausage and meat liver (30 of each) were collected from different restaurants in in Benha city Kaliobia Governorate. Each sample was kept in a separate sterile plastic bag, put in an ice box then transferred to the laboratory under complete aseptic conditions without undue delay for bacteriological examination.

2.2 Bacteriological examination

2.2.1 Preparation of samples [8]

Twenty-five grams of the samples were taken under aseptic condition to sterile Stomacher bag then 225 ml sterile 0.1% peptone water were added, the contents were homogenized at Stomacher for 2 minutes, the mixture was allowed to settled, for 5 minutes at room temperature. The contents were transferred into sterile flask, thoroughly mixed, 1 ml was transferred into separate tube, each containing 9 ml sterile 0.1% peptone water, from which tenth- fold serial dilutions were prepared. The prepared samples were subjected to the following bacteriological examination.

2.2.2 Determination of aerobic plate count following [16]

2.2.3Total coliform count

The technique recommended by [24] using the surface plating method using Violet Red Bile agar medium was applied.

2.2.4 Isolation &identification of E.Coli [7]

From the original dilution, 1ml was inoculated into MacConkey broth tubes supplemented with inverted Durham's tubes. incubated at 37°C for 24 hrs. Loopfuls from positive MacConkey broth tubes were separately streaked onto

plates of (EMB) medium, incubated at 37°C for24 hrs. Suspected colonies were greenish metallic with dark purple center. Suspected colonies were purified, inoculated into slope nutrient agar tubes for further identification.

2.2.4.1 Morphological and biochemical identification according to [32]

2.2.5 Determination of staphylococci count using mannitol agar plates **[24]**

2.2.6 Isolation of s. aureus using paired parker agar [24]

Black, shiny colonies with yellow halo zone around them. Suspected colonies of S. aureus were picked up, purified on Semi-solid agar slopes for morphological examination, biochemical identification, according to [32].

2.2.7 Determination of staph aureus enterotoxin by PCR: according to [37,29,33]

3. Results

The results of bacteriological examination of the examined samples revealed that APC, coliform, Staphylococcal & Staph aureus counts were highest in liver then sausage then kofta then burger. Also, the incidence of co-agulase positive S. aureus is highest in liver then sausage, then kofta, then burger.

Isolation and identification of E. coli in the examined food samples revealed that the incidence of E. coli was highest and of the same percent in both liver and sausage followed by kofta and burger The incidence of Staph. aureus enterotoxins sea, sab, sec and sed were 33.3%, 16.6%, 16.6% and 8.3%, respectively. On the other hand, Citrobacter diversus, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Klebriella ozaenae, Klebriella pneumonia, Proteus mirabilis, Proteus vulgaris and Providencia rettgeri were isolated with varying percentages. In general Enterobacter agglomerans, Enterobacter hafniae and Serratia liquefaciens failed to be isolated from beef burgar samples.

4. Discussion

Ready-To-Eat foods of meat products are highly demanded due to their high biological value, reasonable price, agreeable taste and easily serving. But these constituents can be contaminated by many types of microorganisms due to bad hygienic measurements. Food borne pathogens may constitute health hazard to the consumers. [44]. Therefore, the present study was carried out on cooked samples of burger, kofta, sausage, liver collected from different restaurants in Kaliobia Governorate to evaluate the bacterial quality of them, to evaluate the hygienic health hazard of fast foods contaminated with some food borne pathogens.

Inappropriate storage conditions as street food vendors keep their food at ambient temperatures leading to increase in total bacterial count. While the prepared food should be kept at temperatures below 8^oC and above 63^oC otherwise microorganisms will proliferate [45]. The data shown in Table (1) revealed that the mean values of APC in the examined samples of cooked burger, kofta, sausage, liver were $3.6\times10^4 \pm 0.2\times10^4$, $1.3\times10^5 \pm 0.1\times10^5$, $3.4\times10^5 \pm {}^0.2\times10^5$, $7.8\times10^5 \pm 0.6\times10^5$, respectively. Nearly similar results were obtained by [5], who found that APC in the examined samples of kofta was $1.37X\ 10^5 \pm 1.7X10^4$cfu/g. higher findings were obtained by [1], who found that the APC in beef burger samples was $7.34x10^4 \pm 1.22\ x10^4$ cfu/g. [6] , found that APC in kofta samples was 28.4×10^5 cfu/g. [3] found that the APC in sausage samples was $8\times10^7 \pm 0.37\times10^7$ cfu/g.

Table (1) The results of Aerobic Plate Count(cfu/g) in the examined samples of ready to eat meat products (n=30)

Products	Min.	Max.	Mean± SEM*
Beef burger	0.7×10^4	12.0×10^4	$3.6\times10^4 \pm 0.2\times10^4$
Kofta	0.1×10^5	12.0×10^4	$1.3\times10^5 \pm 0.1\times10^5$
Sausage	0.5×10^5	17.0×10^5	$3.4\times10^5 \pm 0.2\times10^5$
liver	0.8×10^5	28.0×10^5	$7.8\times10^5 \pm 0.6\times10^5$

The results in Table (2) showed that, the incidences of enteric bacteria isolated from the examined samples were Proteus vulgaris 50% was isolated at highest level from beef burger followed by Enterobacter aerogenes 40%, Klebriella ozaenae 30%, Citrobacter freundii & Proteus mirabilis 20% of each and Citrobacter diversus, Enterobacter cloacae, Klebriella pneumonia & Providencia rettgeri 10% of each from beef burger. Moreover, Proteus mirabilis 60%, Klebriella ozaenae 50%, Providencia rettgeri 40%, Citrobacter freundii, Enterobacter aerogenes & Proteus vulgaris 30% of each, Enterobacter cloacae & Klebriella pneumonia 20% 0f each and Citrobacter diversus 10% were isolated from kofta samples. Consequently, Proteus vulgaris 70%, was isolated at highest level from sausage samples followed by Klebriella pneumonia 50%, Enterobacter aerogenes, Klebriella ozaenae & Providencia rettgeri 40% of each, Citrobacter diversus & Proteus mirabilis 30% of each, Citrobacter freundii & Enterobacter cloacae 20% of each and Enterobacter agglomerans 10%. from liver samples Enterobacter aerogenes was isolated at the highest level 70%, followed by Citrobacter freundii, Proteus mirabilis & Providencia rettgeri 50% of each, Enterobacter cloacae, Klebriella pneumonia & Proteus vulgaris 40% of each, Serratia liquefaciens 30%, Citrobacter diversus & Enterobacter agglomerans 20% of each and Klebriella ozaenae 10%. Such organisms were previously isolated from fast food by [11,21,12] who isolated these members of Enterobacteriaceae from the examined samples of ready-to-eat kofta.

Table (2) Incidence of Enteric bacteria isolated from the examined samples of ready to eat meat products (n=30)

Identified Bacteria	Beef burger		Kofta		Sausage		Liver	
	No.	%	No.	%	No.	%	No.	%
Citrobacter diversus	1	10	1	10	3	30	2	20
Citrobacter freundii	2	20	3	30	2	20	5	50
Enterobacter aerogenes	4	40	3	30	4	40	7	70
Enterobacter agglomerans	-	-	-	-	1	10	2	20
Enterobacter cloacae	1	10	2	20	2	20	4	40
Enterobacter hafniae	-	-	1	10	-	-	-	-
Klebriella ozaenae	3	30	5	50	4	40	1	10
Klebriella pneumoniae	1	10	2	20	5	50	4	40
Proteus mirabilis	2	20	6	60	3	30	4	50
Proteus vulgaris	5	50	3	30	7	70	3	40
Providencia rettgeri	1	10	4	40	4	40	5	50
Serratia liquefaciens	-	-	-	-	1	10	3	30

Data presented in Table (3) showed that, the mean values of coliform count in the examined cooked burger, kofta, sausage, liver were $1.6 \times 10^3 \pm 0.1 \times 10^3$, $3.0 \times 10^3 \pm 0.2 \times 10^3$, $4.1 \times 10^3 \pm 0.3 \times 10^3$, $7.0 \times 10^3 \pm 0.5 \times 10^3$ respectively. The current results agree with those recorded by [12,1]. However, higher findings were obtained by [17,38].

Results in Table (4) revealed that the incidences of *E. coli* in beef burger & kofta samples and sausage & liver samples have the same incidence of occurrence 16.7%, 33.3%, respectively. These results came in accordance with those obtained by [4], but higher than [41,34], and lower than [47,13,18,27,2]. In general, heat treated foods must be free from E. coli [19]. The detection or even low number of E. coli in foods constitutes a public health hazard [23].

Table (3) The results of total Coliform counts (cfu/g) in the examined samples of ready to eat meat products (n=30)

Products	Min.	Max.	Mean± SEM*
Beef burger	0.7×10^4	12.0×10^4	$3.6 \times 10^4 \pm 0.2 \times 10^4$
Kofta	0.1×10^3	9.4×10^3	$3.0 \times 10^3 \pm 0.2 \times 10^3$
Sausage	0.4×10^3	14.0×10^3	$4.1 \times 10^3 \pm 0.3 \times 10^3$
liver	0.9×10^3	36.0×10^3	$7.0 \times 10^3 \pm 0.5 \times 10^3$

Table (4) Incidence of pathogenic E. coli isolated from the examined samples of ready to eat meat products (n=30)

products	No.	%
Beef Burger	3	16.7 %
Kofta	3	16.7%
Sausage	6	33.3%
Liver	6	33.3%
total	18	100%

Table (5) indicated that the mean values of Staphylococcal count in the examined cooked burger, kofta, sausage, liver were 9.0×10^{٢} ± 0.8×10^{٢}, 2.3×10^{3} ± 0.2×10^{3}, 4.3×10^{3} ± 0.4×10^{3}, 5.9×10^{3} ± 0.4×10^{3} respectively. The current results of kofta were nearly similar to those obtained by [34] 1.13 $x10^{3}$ cfu/g. Meanwhile, they were lower than those obtained by [13] $6.91x10^{3}$ cfu/g and [48] 5.41×10^{3} cfu/g.

Table (5) The results of Staphylococci counts (cfu/g) in the examined samples of ready to eat meat products (n=30)

Products	Min.	Max.	Mean± SEM*
Kofta	0.3×10^{3}	8.0×10^{3}	2.3×10^{3} ± 0.2×10^{3}
Sausage	0.9×10^{3}	9.0×10^{3}	4.3×10^{3} ± 0.4×10^{3}
liver	1.0×10^{3}	20.0×10^{3}	5.9×10^{3} ± 0.4×10^{3}

Furthermore, the results obtained in Table (6) revealed that Staph. aureus were isolated from beef burger, kofta, sausage and liver sandwiches with an incidence of 14.3% with a mean value of 4.8×10^{2} ± 0.3×10^{2} ,19% with a mean value of 6.4×10^{2} ± 0.8×10^{2}, 28.6% with a mean value of 1.6×10^{3} ± 0.1×10^{3} and 38.1% with a mean value of 2.2×10^{3} ± 0.1×10^{3}, respectively. Nearly similar results were recorded by [31,22]. These results were disagreed with those of [28,35,26] who found that all examined kofta, sausage samples were free from S. aureus. The problem of Staph. aureus as contaminants in the food supply remains significant on a global level as it is an important causative agent of food poisoning outbreaks worldwide [10]. Although food handlers are usually the main source of food contamination in food poisoning outbreaks, equipment and environmental surfaces can also be sources of contamination with Staph. aureus [42].

The obtained results in Table (7), showed that the incidences of Staph. Aureus enterotoxins sea, sab, sec and sed in the examined samples were 33.3%, 16.6%, 16.6%, 8.3%, respectively. The current results nearly similar to those recorded by [46,20]. Time and temperature abuse of a food product contaminated with enterotoxigenic Staphylococci can result in formation of enterotoxin, which produce foodborne illness. [9]. This enterotoxin is heat stable. Even if the food is heated before eating, the poison in the food will cause illness although the heat has killed the bacterial cells [14].

Finally, the present study proved that ready to eat meat products are considered public health hazard, due to the presence of aerobic bacteria, Enterobacteriaceae, coliforms, E. coli, Staphylococci, Staph. aureus enterotoxins.

Table (6) Statistical analytical results of Staph. aureus count (cfu/g) in the examined samples of ready to eat meat products (n=30)

Products	+ve Samples		Min.	Max.	Mean± SEM*
	N0.	%			
Beef burger	3	14.3	1.0×10^{2}	9.0×10^{2}	4.8×10^{2} ± 0.3×10^{2}
Kofta	4	19.0	2.0×10^{2}	10.0×10^{2}	6.4×10^{2} ± 0.8×10^{2}
Sausage	6	28.6	0.5×10^{3}	4.0×10^{3}	1.6×10^{3} ± 0.1×10^{3}
liver	8	38.1	0.6×10^{3}	7.0×10^{3}	2.2×10^{3} ± 0.1×10^{3}

Table (7) The incidence of occurrence of enterotoxin of staph aureus isolated from the examined samples (n=12) of meat products by using Multiplex PCR

Sample	sea	seb	sec	sed
3	-ve	-ve	-ve	-ve
4	-ve	-ve	-ve	-ve
5	-ve	-ve	+ve	-ve
6	-ve	-ve	-ve	-ve
7	+ve	-ve	-ve	-ve
8	-ve	-ve	-ve	-ve
9	-ve	-ve	-ve	-ve
10	+ve	+ve	-ve	-ve
11	-ve	-ve	-ve	+ve
12	+ve	+ve	+ve	+ve
13	+ve	-ve	-ve	-ve
14	-ve	-ve	-ve	-ve

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