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Original Article

Evaluation of the Microbial Quality of Food Served in a University Hospital in Alexandria

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Abstract

Background: Foodborne illness is a serious health-related problem especially in hospitalized patients.
Objective(s): The present study aimed to evaluate the bacterial contamination of meals served in a university hospital in Alexandria, Egypt.
Methods: This cross-sectional study covered 280 samples of processed and non-processed food that were randomly taken from various wards, from a university hospital in Alexandria. All samples were exposed to aerobic plate count using pour plate method. Multiple tube dilution approach was used to detect and enumerate total coliforms, fecal coliforms, and *Escherichia coli (E. coli)*.
Results: The total plate count (TPC) of the 280 samples ranged from $2.2x10^2$ to $4.4x10^{11}$ CFU/g. Tomatoes had the highest mean value $9.98x10^9$ CFU/g, while bread exhibited the lowest mean value 2.5 $x10^2$ CFU/g. The fecal coliform (FC) count for processed and non-processed food showed unsatisfactory results in 24.3% and 42.0 % (reviewer 2 comment A6) respectively. *E. coli* were detected in 7.1 % of processed and 30.6% of non-processed samples.
Conclusion: The considerable unsatisfactory level of *E. coli* in some of the tested samples is augmenting the need to improve food preparation, handling, storage, and distribution in the hospital.

Keywords: Microbial quality of food, University hospital, Coliforms, E. coli

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INTRODUCTION

Despite the noticeable development in therapeutic care and food machinery, food-borne illnesses are still a major public health problem in both developing and developed countries. Food-borne illnesses represent 11% of food poisoning, and over 90% of cases are of bacterial origin. Hospitalized patients are more liable to suffer serious complications.(1)

The aim of hospital food service is to supply in-patients with safe nourishing meals appropriate to their particular health status.(2)

Outbreaks of foodborne infections in hospitals are avoidable, but usually occur due to poor sanitation circumstances in the kitchens, inattention, and loss of guidance of food producer. The specific crisis of infected meal in hospitals is that such food is introduced to users in poor health.(3, 4)

The Centers for Disease Control and Prevention (CDC) demonstrates that every year, 48 million persons get a foodborne illness, of whom 128,000 are hospitalized, and 3,000 died.(5) The Hazard Analysis Critical Control Point (HACCP) system is becoming progressively demanded in food regulation. HACCP must be applied by an entire food safety system starting from the raw materials, production, storage, transportation, allocation, and distribution.(6) The HACCP program must be fitted to the particular individual product and processing line. A successful HACCP program should be designed by following the overall system in mind. It is also important that staff members are involved in the outlining and pattern phases of the HACCP plan.(7)

The aim of this study was to survey the bacteriological contamination in served processed and non-processed food in a university hospital in Alexandria, Egypt.

METHODS

The present cross-sectional study was carried out at a university hospital in Alexandria, Egypt, starting from September 2018 to April 2019. It involved 280 samples

(140 processed and 140 non-processed foods) collected randomly from different wards in the hospital. Samples were transported to the High Institute of Public Health (HIPH) microbiology laboratory in a cool box where they were tested immediately.

Samples included 140 processed (53 Rice, 41 Meat, 34 Chicken, and 12 Bread), as well as 140 non-processed meals (59 Cucumber, 50 Tomato, 10 Date, 12 Grape, and 9 Banana). The main dishes were Egyptian classical meals prepared with either red meat or chicken. The salads and fruits were put as raw. The rice was prepared by boiling.

About 50 grams from each of the different food samples were collected aseptically in sterile containers labeled with the type of sample, date, time of collection, and transported immediately to the HIPH laboratory.

1. Preparation of sample homogenate

From the above samples, 25 g of each was mixed with 225 ml sterile peptone water, in a sterile plastic bag of the stomacher, and homogenized in the stomacher for 2-3 minutes.

2. Microbiological Examination[8]

2.1. Determination of total plate count (TPC) using the pour plate method

Ten-fold dilutions were prepared from the sample homogenate in diluent buffer peptone water to get 10^{-2}, 10^{-3}, 10^{-4} dilutions. One ml of each dilution was pipetted into a sterile petri dish (duplicate plates) under complete aseptic conditions. Twenty ml of melted sterile plate count agar (45°C) **(**Oxoid Code: BO0195) were then poured into each petri dish and the contents were mixed thoroughly by rotating the plate several times. Upon solidification of the media, the plates were inverted and incubated at 37°C for 48 hours. After incubation, the colonies were counted using the Quebec counter. Control plates were included. Plates having colony count between 25 and 250 [9] colonies were chosen. The average number of colonies/plate was multiplied by the dilution factor and recorded as CFU/g.

2.2. Enumeration of the total coliforms, fecal coliforms, and E. coli using multiple tube dilution method

2.2.1. Presumptive test

Three tubes of lauryl tryptose sulfate (LST) (Oxoid Code: CM0451) broth per dilution were inoculated with one ml of the previously prepared 1:10, 1:100 and 1:1000 dilutions. Tubes contained inverted Durham's tubes for gas detection. The tubes were then incubated at 37°C. All tubes were observed for gas production in the inverted Durham's tubes after 24 hours. Negative tubes were re-incubated for an additional 24 hours. All LST tubes showing both turbidity and gas within 48 hours were considered as presumptively positive for total coliforms (TC).

2.2.2. Confirmed test

Three loopful of presumptive positive tubes were inoculated in brilliant green lactose bile broth (BGLBB) (Oxoid Code: CM0031). All tubes were shaken on a vortex mixer. A set of BGLBB tubes was incubated at 35-37 °C for 24-48 hours within 30 minutes after inoculation for the detection of TC. Another set of BGLBB tubes was incubated at 44 ± 0.2°C for 24 hours in a circulating covered water bath for the detection of fecal coliforms (FC). The tubes were submerged in the bath so that the water level was above the highest level of the medium. BGLBB tubes showing turbidity and gas at 37°C after 48 hours were recorded as positive for TC. While, BGLBB tubes showing turbidity and gas production after 48 hours at 44 ± 0.2°C were recorded as positive for FC.

2.2.3. Complete test for *E. coli*

Positive BGLBB tubes from FC were streaked on eosin methylene blue (EMB) (code: CM0069) agar plates and incubated for 24 hours at 37°C. Positive blue-black colonies with a green metallic sheen were identified as *E. coli*.

2.3. Isolation, identification and enumeration of *E. coli* [8]

All typical colonies of *E. coli* from EMB were identified by Gram stain, and biochemical tests using Triple sugar iron (TSI) (Acid butt, acid slant, without H_2S gas pocket or cracking of the agar), indole, and methyl red positive, citrate and urease negative tests.

Most probable number (MPN) of *E. coli was* calculated based on the proportion positive tubes in 3 successive dilutions, which have been shown to contain *E. coli.*

The degree of microbial contamination (satisfactory, borderline, and un-satisfactory) for all samples was assessed using the guidelines of ready to eat food according to Public Health Laboratories guidelines (PHLS) [9] (Table 1).

Table 1: Guidelines for ready to eat (RTE) foods according to Public Health Laboratories guidelines (PHLS) guidelines

Hygiene indicator organism	Guidelines for RTE processed and non-processed samples		
	Satisfactory	Borderline	Unsatisfactory
Total Plate Count (CFU/g)	$<10^6$	10^6 - $<10^7$	$\geq 10^7$
Thermotolerant coliforms (Fecal coliforms) (MPN/g)	$<10^2$	10^2 - 10^4	$> 10^4$
E. coli (MPN/g)	<20	20-100	> 100

MPN: Most probable number

RESULTS

Out of 140 processed samples, all (100%) showed aerobic bacterial growth in TPC, 130 (92.8%) showed TC, 103 (73.5%) showed FC, of which 74 were *E. coli*. While, out of 140 non-processed samples, all (100%) showed growth in TPC, 138 (98.5%) were positive for TC, 114 (81.4%) were positive for FC and 105 (75%) grew *E. coli* (Table 4). The mean count of TPC, TC, FC, and *E.* coli are demonstrated in Table 3.

Table 2: The total plate count, total coliforms, fecal coliforms, and *E. coli* for all studied samples

Type of sample	Min. – Max.	Mean ± SD	Median
Processed samples			
Total plate count (CFU/g)	$2.2x10^2 – 2.65x10^9$	$1.6x10^6 \pm 2.70x10^8$	$2.20x10^8$
Total Coliforms (MPN/ g)	$0.0 – 1.1x10^3$	$2.3x10^2 \pm 4.8x10^2$	$2.1x10^2$
Fecal Coliforms (MPN/ g)	$0.0 – 1.1x10^3$	$1.3x10^2 \pm 1.7x10^2$	5.4x10
E. *coli* (MPN/ g)	$0.0 – 4.6x10^2$	1.1x10 ± 8.3x10	0.0
Non-processed samples			
Total plate count (CFU/g)	$6.7x10^2 - 4.39x10^{11}$	$4.9x10^9 \pm 4.6x10^{10}$	$1.2x10^6$
Total Coliforms (MPN/ g)	$0.0 - 1.10x10^3$	$6.4x10^2 \pm 4.11x10^2$	$4.60x10^2$
Fecal Coliforms (MPN/ g)	$0.0 - 1.10x10^3$	$4.50x10^2 \pm 5.5x10^2$	$2.7x10^2$
E. *coli* (MPN/ g)	$0.0 – 1.1x10^3$	$3.05x10^2 \pm 5.91x10^2$	1.4x10

Table 3: The total plate count, total coliforms, fecal coliforms and *E. coli* count at 37ºC and 44ºC for all studied samples

Type	Number of samples	37 ºC temperature		44 Cº temperature	
		Total plate count (CFU/g)	Total coliforms (MPN/g)	Fecal coliforms (MPN/g)	E. *coli* (MPN /g)
Processed food	**140**				
Rice					
Mean ± SD.	53	$3.2x10^5 \pm 6.0x10^5$	$3.2 x10^2 \pm 4.46 x10^2$	$1.7 x10^2 \pm 2.67 x10^2$	$1.1 x10 \pm 2.26 x10^2$
Meat					
Mean ± SD.	41	$2.64x10^5 \pm 4.21x10^5$	$4.6 x10^2 \pm 1.6 x10^2$	$2.37x10^2 \pm 3.13 x10^2$	$1.2 x10^2 \pm 6.45 x10^2$
Chicken					
Mean ± SD.	34	$4.5x10^4 \pm 8.68x10^4$	$2.8 x10^2 \pm 2.7 x10^2$	$1.3 x10^2 \pm 1.3 x10^2$	2.0 x10 ±4.5 x10
Bread					
Mean ± SD.	12	$2.46 x10^2 \pm 3 x10$	2.0 ± 1.73	0.0 ± 0.0	0.0 ± 0.0
Non-processed food	**140**				
Cucumber					
Mean ± SD.	59	$1.8x10^6 \pm 1.51x10^6$	$3.1x10^2 \pm 3.9x10^2$	$2.9\times10^2 \pm 4.1\times10^2$	$2.01 x10^2 \pm 3.12 x10^2$
Tomato					
Mean ± SD.	50	$9.9x10^9 \pm 6.62x10^{10}$	$4.7x10^2 \pm 3.8x10^2$	$3.9x10^2 \pm 1.1\times10^3$	$3.9 x10^2 \pm 3.0 x10^2$
Date					
Mean ± SD.	10	$9.5x10^5 \pm 1.14x10^6$	$1.9x10^2 \pm 0.0$	$1.2\times10^2 \pm 1.2\times10^2$	$2.2 x10^2 \pm 4.26 x10^2$
Grape					
Mean ± SD.	12	$2.2x10^3 \pm 3.4x10^2$	$1.7x10^2 \pm 6.1x10^2$	$8.7\times10 \pm 1.6\times10^2$	$1.2x10^2 \pm 6.26 x10^2$
Banana					
Mean ± SD.	9	$1.1x10^3 \pm 6.1x10^2$	7.30 ± 5.23	0.0 ± 0.0	0.0 ± 0.0

MPN: Most probable number.

The mean for TPC, TC, FC and *E. coli* at 37 ºC and 44ºC for all samples is demonstrated in table 3. Concerning the non-processed food, tomatoes were the highest in mean count of TC (4.68×10^2), FC (3.92×10^2), and E. *coli* (3.9×10^2). While banana showed the lowest TC mean (7.30), with no FC or *E. coli* recorded. Regarding the processed samples, the rice showed the highest mean ACC (3.18×10^5) and the meat recorded the highest TC mean (4.56×10^2 MPN/g) as well as *E. coli* mean (1.23×10^2 MPN/g). The lowest TC was observed with bread (2.0) with no reported *E. coli*. According to PHLS guidelines, the non-processed samples reported higher number of unsatisfactory results than the processed samples. Fifty processed samples and 101 non-processed samples showed significant unsatisfactory results ($\geq10^7$ CFU/g) in) TPC (p <0.001). Additionally, the non-processed food samples had statistically a higher percentage of unsatisfactory samples for both FC (59/140) and *E. coli* (43/140) than the processed samples (p <0.005, p <0.001 respectively) (Table 4).

Table 4: Comparison between the processed and non-processed samples according to satisfactory results according to Public Health Laboratories guidelines (PHLS)

	Processed (n = 140)		Non-processed (n = 140)		χ^2	*p*
	No.	%	No.	%		
Total plate count (TPC)						
Satisfactory $<10^6$	83	59.3	22	15.7		
Borderline $\geq10^6$ - $<10^7$	7	5.0	17	12.1	56.830*	<0.001*
Unsatisfactory $\geq10^7$	50	35.7	101	72.1		
Fecal coliforms (FC)						
Satisfactory $<10^2$	73	52.2	52	37		
Borderline 10^2 -$<10^4$	33	23.5	29	21	10.506*	<0.005*
Unsatisfactory $> 10^4$	34	24.3	59	42		
E. coli						
Satisfactory <20	98	70.0	73	52.2		
Borderline 20 - <100	32	22.9	24	17.2	25.345*	<0.001*
Unsatisfactory ≥100	10	7.1	43	30.6		

DISCUSSION

The present study aimed to estimate the microbial contamination of food served in a university hospital in Alexandria. Assessing TPC result requires knowledge of the food and processing conditions. Indicator organisms indicate that contamination has developed due to inappropriate processing or post-processing (cross-contamination from food contact surfaces, raw products or food handlers). Low cooking temperature and time managing may also be a sharing cause [10, 11]. In the present work, we confirmed the presence of microbial contamination at a rate of (100%) of all processed and non-processed samples. All samples yielded growth of aerobic bacteria with varying densities. It has been documented that aerobic organisms reflect the exposure of samples to any contamination and generally the presence of supportive circumstances for microorganism's reproduction. Similar results were reported by Khamis and Hafez [12].

The means of TPC for processed and non-processed samples in the current work were $1.6x10^6$ and $4.9x10^9$ respectively. The acceptable limit of fresh vegetables by some countries for export purposes should not exceed 4.9×10^6 CFU/g.[13] The TPC of the 140 non-processed food samples examined in this study showed a wide range of microbial load $6.7\times10^2 - 4.39\times10^{11}$ CFU/g. This high count was comparable to results for Abaza et al.,[14] (5.8×10^6) and Kubheka et al.,[15] in South Africa. Similarly, Faour-Klingbeil et al., in Lebanon reported that the mean levels ranged from 7.9×10^2 to 2.4×10^7 CFU/g.[16] Furthermore, by following the present results, Khalil and Gomaa in Egypt recorded a wide range of TPC for conventional vegetable samples ($4.3x10^3 - 1.5\times10^7$ CFU/g).[17]

This work included 280 samples 140 processed food samples distributed as (53 Rice, 41 Meat, 34 Chicken, and 12 Bread) and 140 non-processed food samples distributed as (59 Cucumber, 50 Tomato, 10 Date, 12 Grape, and 9 banana). The categorization of food allowed us to recognize which type of food more dangerous and may cause health problems and to give this type of food special attention. Therefore, food served in hospitals must be routinely checked and followed up to prevent and minimize foodborne illness/collective food poisoning especially among weakened populations. According to PHLS guidelines for the examined 140 processed and 140 non-processed samples in this study, 35.7%, 72.1% were unsatisfactory, respectively regarding TPC. Nearly similar results were reported by Hannan et al., in Pakistan [18].

Microbiological analysis results of the variable food categories showed that vegetables and fruits class was the most contaminated one 72.1% which is comparable to Zbadi et al.,[19] 32% and Aycicek et al.,[20] 31.4%. Other studies noted high rates of contamination in salads 93% and fruits 65%.[9,13,21] For processed foods, rice 3.2×10^5 CFU/g and meat 2.6×10^5 CFU/g showed high count in the present study. In contrast, Akindele and Ibrahim et al., showed much lower count for rice 1.0×10^2 CFU/g and meat 6.0×10^4 CFU/g.[21] While in study of Khater et al., the range was (5.7×10^3 to 9.77 10^6) the high bacterial count would indicate that they were contaminated during, after cooking or during handling procedure and that demonstrate overall lack of hygiene.[22]

This study shows a high level of Total Coliforms in non-processed food 6.4×10^2 MPN/g and processed food $2.3x10^2$ MPN/g. Similar results were reported by Khater et al., 6.9×10 to 4.8×10^3 MPN/g.[22] The mean values of FC for processed and non-processed samples were 1.3×10^2 MPN/g and 4.50×10^2 MPN/g respectively. The highest count in processed food was for meat 2.4×10^2 MPN/g and for non-processed was for tomatoes 3.9×10^2 MPN/g. Results of FC in the Abaza et al.,[14] study exhibited the presence of fecal contamination in 90.1% of the examined fresh vegetable samples. FC mean values ranged between 8.5×102 and 1104 MPN/100 g, whereas tomato samples had the lowest mean 850 MPN/100 g. Heavy contamination with FC 4.0×103 – 9.3×108 MPN/g was

also observed in a survey that was carried out in Ghana on some vegetables cultivated with poor-quality irrigation water. In Ethiopia, Weldezgina et al., stated that MPN of total and FC and their overall range in vegetables was (865.3 – 1036.0) and (524.0 – 716.0 MPN/100 ml) respectively [23]. The mean values for processed and non-processed samples for *E. coli* were 1.1×10 and 3.1×10^2 respectively with the highest count in meat (1.23×10^2). Comparing the current results to that reported by Rodriguez et al.,[24] *E. coli* was found in salads samples (6.3%) while no *E. coli* contamination was found in cooked meat products. In Ismail et al. study, *E. coli* was detected in 17.3% of the tested samples [25]. The presence of *E. coli* in heat-processed food refers to post-processing contamination. The presence of even low numbers of *E. coli* in the examined samples reveals a public health hazard.

CONCLUSION AND RECOMMENDATIONS

The considerable unsatisfactory level of *E. coli* in some of the tested samples is augmenting the need to improve food preparation, handling, storage, and distribution processes in the hospital.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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