

Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

Original article

Microbiological study of some commonly used foods and drinks in Tanta Al Gharbia Governorate

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ARTICLE INFO

Article history:

Received 7 March 2022

Received in revised form 6 April 2022

Accepted 11 April 2022

Keywords:

Contamination of food and drink

Foodborne illness

Enterotoxins

Multiplex PCR

ABSTRACT

Background: Food borne illness represents a major public health problem associated with high morbidity and mortality rates. **Methods:** One hundred samples (foods and drinks) were collected in sterile stomacher bags, Bacterial isolation, colony count, antimicrobial susceptibility testing, and detection of *Staphylococcus aureus* (*S.aureus*) enterotoxin genes by multiplex PCR were done. **Results:** The current study revealed that the Gram-negative bacilli were the most frequent isolated organisms representing (63.3%) followed by Gram positive bacteria (34.5%) and fungi were the least found (2.2%). Among Gram negative bacilli, *Klebsiella* was the most frequently isolated bacteria, among Gram positive bacteria, *Staphylococci* were the most frequently isolated bacteria, all isolated fungi were *Candida* species. Out of 16 identified *S. aureus* strains recovered from the examined samples, 6 isolates were proved to be enterotoxigenic. One isolate carried Staphylococcal enterotoxin C (SEC) gene, two isolates carried both Staphylococcal enterotoxin A (SEA) genes and Staphylococcal enterotoxin B (SEB) gene, and three isolates carried SEA, SEB, and SEC genes. None of the isolates contained SED gene. **Conclusion:** Based on the findings of this study, food borne illness represent a major public health problem in Tanta.

Introduction

Food-borne illness have a very bad effect on public health and negatively affect the economy of the countries and affect their work ability ranging from less efficient work to complete inability to work [1], according to the Center for Disease Control and Prevention (CDC) report of 2018, globally; 48 million people get sick from a food borne illness, 128.000 are hospitalized, and 3000 die in each year [2], with the highest rates of morbidity and mortality in developing countries [3]. So it needs many researches on food and drinks for proper early diagnosis and treatment of cases [4]. Food-

borne illness is acute illnesses resulting from recent consumption of contaminated food or drinks. The clinical picture of the illness are due to the presence of contaminating bacteria, viruses, parasites and/or their toxins [5]. The most common of food-borne illness symptoms are nausea, vomiting, abdominal cramps and diarrhea. Fever, dehydration and neurovascular symptoms are associated with some food borne illness [6]. Food-borne illness outbreaks are defined as two or more cases of a similar food-borne illness due to the consumption of a common food and drink [7]. The most common causative bacteria of food borne illness are *Salmonella*,

DOI: 10.21608/MID.2022.126007.1255

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Clostridium perfringens, *Campylobacter*, *S. aureus*, *Escherichia coli* (*E. coli*), and *Bacillus cereus*, the most common causative viruses are Norwalk virus, rota virus, hepatitis A virus, and hepatitis E virus, also fungi and parasites can cause food borne illness [8]. Food-borne illness due to *Salmonella*, *Norwalk virus*, *Campylobacter*, *Staph. aureus*, *Toxoplasma gondii* and *E. coli* infections associated with patient hospitalization [9]. Incubation period of food borne illness usually ranges from few hours after consumption of contaminated food or drink to several days [10]. In Egypt the most common food associated with food borne illness are Koshari (Mix of lentils, rice, macroni and tomatoes), Fool (boiled beans) and Tamia (minced beans and vegetables). In addition to the well-known drinks like sugar cane juice and liquirice. Other types of food include eggs, poultry, meat, unpasteurized milk, cheese, raw or unwashed fruits and vegetables, nuts, juices [11]. Many factors affect the occurrence of food borne illness such as improper cooking of food, indirect contamination of food or drinks by hands of food handlers or through different insects like flies, cockroaches or through rats [12]. *Staphylococcus aureus* is a common bacterial cause of food borne illness due to the production of wide varieties of heat-stable enterotoxins. About 50% of *S. aureus* strains can produce enterotoxins. They are classified into five main classical types including SEA, SEB, SEC, SED and staphylococcal enterotoxin E (SEE). All of them are of human origin except enterotoxin D is of animal origin [13]. This study aimed to examine different samples of foods and drinks in Tanta for the most common human pathogens that contaminate food and drink and to do anti-microbial susceptibility of the isolated pathogens.

Materials and Methods

The present study was carried out at The Clinical Microbiology and Immunology Department, Faculty of Medicine, Tanta University and Central Research Laboratory of Ministry of Health, Tanta city. It included 100 samples of different food and drink bought during the period of research from March 2020 to February 2021 from different street vendors, roadside cafeterias, restaurants, open markets and supermarkets in Tanta city. Ethical approval for this study was provided by Ethics and Research Committee, Faculty of Medicine, Tanta University. A code number was put for each sample for adequate provision to maintain confidentiality of the data. The inclusion criteria were the samples which were either processed or

cooked. Exclusion criteria were: Samples not examined in the same day of collection, samples which were not well preserved, samples which were late for transportation.

Sample collection and transportation

The solid samples were collected using sterile plastic tools in sterile plastic bags.

Fluid samples were collected with sterile plastic syringes that were used for immediate inoculation on solid or fluid media. Samples were labelled and recorded in data entry sheet which included the location, date, time of collection, type of sample and observations on preservation and handling (handlers and tools) and transferred to the laboratory as soon as possible for processing. The samples included various types of food and drink such as Koshri, Koskosi, Fool, Tamia, Sugarcane juice, eggs, unpasteurized milk, Yoghurt, cheese, unwashed or raw fruits, vegetables, nuts, juices, fishes either fried, boiled, grilled, roasted or cooked fish, chicken and meat from different selling outlets in Tanta city. About 100 grams of each sample were collected, labeled by code numbers each corresponding to a specific type of sample, place and date of collection. Then it was transported in an ice box to the laboratory in Medical Microbiology and Immunology Department for processing within 1 hour after collection [14].

Sample preparation and bacterial count

Ten gm of each sample was aseptically weighed into sterile stomacher bag and homogenized after the addition of 90.0 mL of sterile peptone water. Masticator silver blender at 200 rpm for 1-2 minutes at central research laboratory of ministry of health to prepare the initial dilution 1:10 (10⁻¹). One milliliter of the homogenate was added to 9 mL of sterile peptone water in a test tube, labelled 10⁻² dilution and serially diluted to five other test tubes labelled 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶ and 10⁻⁷. One hundred microliters of each of the diluted sample was spread plated on Nutrient agar. The plates were incubated aerobically for 24 h at 37 °C. All discrete colonies were counted where possible and expressed as the log 10 of colony forming units (CFU) per gram⁻¹ [15].

Culture and biochemical reactions

A loopful of each homogenized sample were cultured on MacConkey, blood, mannitol salt, bile esculin agar and then Gram stain smears were made after culture to avoid contamination of the samples and examined microscopically. Enrichment of part

of homogenized sample was done on selenite broth for 24 h before cultivation on xylose lysine deoxycholate (XLD) for *Salmonella* and *Shigella* species isolation. Anaerobic culture was done on anaerobic gas pack system and incubated for 48 hour. Colonies identified by colony morphology, Gram staining, and biochemical reactions were oxidase test, citrate utilization test, indole test, sugar fermentation tests, triple sugar iron agar (TSI agar), coagulase test, catalase test.

Antibiotic sensitivity testing

Antimicrobial susceptibility of the isolates was determined by modified Kirby Bauer disc diffusion method using antibiotics discs on Mueller Hinton agar plates according to clinical and laboratory standard institute (CLSI) guideline [16]. Gram positive organisms were tested against the following: Penicillin G (10 units), oxacillin (5µg)/ampicillin (10 µg), vancomycin (30 µg), linezolid (30 µg), ceftazidime (30 µg), cotrimoxazole (1.25/23.75 µg), erythromycin (15 µg), ciprofloxacin (5µg)/ levofloxacin (5 µg), gentamicin (20/10 µg). Gram negative organisms were tested against the following: Meropenem (10 µg), amikacin (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), tobramycin (30 µg), amoxicillin clavulanic acid (20/10 µg), cotrimoxazole (1.25/23.75 µg), ceftazidime (30 µg), colistin (10µg)

Molecular detection of *Staphylococcus aureus* enterotoxin genes (SEA, SEB, SEC, SED)

DNA extraction was done according to manufactures instructions (Genaid co.), PCR amplification BY 2 X easy Taq PCR super mix. Detection of amplified DNA by gel electrophoresis and ultra-violet light transillumination.

DNA extraction

1. Culture cell sample preparation

- Cell was transferred (up to 1x10) to a 1.5 ml microcentrifuge tube.
- Then centrifuged to 5 minutes at 300 x g.
- The supernatant was discarded then resuspended cells in 200 ul of phosphate buffered saline by pipette.
- 20 ul of proteinase k was added then mixed by pipetting.
- Incubated at 60 C for 5 minutes.

2. B cell lysis

- 200 ul of gel sample buffer (GSB) was added then mixed by shaking vigorously

- Incubated at 60 C for 5 minutes, the tube was inverted every 2 minutes.
- The required volume of Elution Buffer was transferred during incubation (200 ul / sample) to 1.5 ml micro centrifuge tube to heat to 60 (for step DNA Elution).

3. RNA removal step

- For RNA-free g DNA following GSB Buffer addition and 60 c incubation.
- 5 ul of RNase A was added (50 mg 1ml) and mixed by shaking vigorously and incubated at room temperature for 5 minutes to ensure efficient RNA degradation.

4. DNA Binding

- 200 ul of absolute ethanol was added to the sample and mixed by shaking vigorously for 10 seconds.
- If precipitate appeared break it up as much as possible with pipette.
- G.S column was placed in a 2 ml collection tube.
- all of the mixture was transferred including any in soluble precipitate to G.S column then centrifuged at 14-16,000 x g for 1 minute.
- Following centrifugation.
- If the mixture did not flow through G.S column membrane increase the centrifuge time until it passes to a new 2 ml collection tube.

Note

It is important that the lysate and ethanol are mixed thoroughly to yield a homogenous solution.

5. Wash

- 400 ul of W1 Buffer was added to GS column.
- Centrifuged at 14-16,000 xg for 30 seconds.
- The flow through was discarded.
- The GS column was placed back in the 2 ml collection tube.
- 600 ul of wash Buffer was added (make sure absolute ethanol was added)
- Centrifuged at 14-16,000 xg for 30 seconds then the flow through was discarded.

- The GS column was placed back in the 2 ml collection tube.
- Centrifuged again for 3 minutes at 14-16,000 x g to dry the column matrix.

6. Elution

- The dried GS column was transferred to a clean 1.5 ml micro centrifuge tube.
- 100 ul of pre-heated Elution Buffer was added.
- TE Buffer or water into the center of the column matrix.
- Let stand for at least 3 minutes to allow Elution Buffer.
- TE Buffer or water to be completely absorbed.
- Centrifuge at 14-16,000 x g for 30 second to elute purified DNA.

PCR amplification BY 2 X easy Taq PCR super mix

- Easy Taq PCR super mix was already-to- use mixture of easy taq DNA polymerase, DNTPS, and optimized buffer.
- The super mix was provided at 2 x concentration and used at 1 x concentration by adding template, primer and H₂O.
- PCR product were not suitable for PAGE.
- Extension rate was about 1-2 Kb/min.
- Template-independent was generated at the 3 ends of the PCR product.
- PCR products were cloned into PEASY-T vectors.
- Genomic DNA fragment was amplified up to 4 Kb.

Thermal cycling condition

- 94° C 2-5 min
- 94° C 30 sec
- 50-60° C 30 sec
- 72° C 1-2 Kb/ min
- 72° C 5-10 min

Detection of PCR amplification product using gel electrophoresis and ultra-violet light transillumination

- The amplified samples were then run in on 2% agarose gel in presence of a DNA marker.
- Using gel electrophoresis and visualized on a UV transilluminator to detect presence of amplified product and to type the ABC genotypes.

Technique

1. Preparation of the agarose gel

2% agarose gel was prepared using Pharmacia gel electrophoresis apparatus according to the following:

- a. The gel was cleaned and dried before use and the gel comb was placed in position in the gel tray then the tray was placed on a horizontal surface.
- b. One gram agarose was added to 50 ml of the ready to use (Trisacetate with EDTA) TAE buffer in a conical to prepare 2% agarose.
- c. Agarose was dissolved by heating using hot plate for 4 minutes.
- d. The agarose was then left to cool between 50-60 C followed by the addition of 5 ul ethidium bromide and proper mixing.
- e. This solution was then poured into the gel tray and left to dry for 30 minutes at room temperature.
- f. Enough ready to use TAE buffer to cover the gel surface to a depth of least 1 mm was poured into the apparatus.
- g. The gel comb was carefully removed.

The apparatus was then ready for loading of samples and performance of electrophoresis

2. Sample preparation and loading

- Samples were prepared for loading by adding 2 ul loading buffer to 7 ul of the PCR reaction mixture.
- That PCR reaction mixture were slowly and carefully loaded into the sample wells using an automatic micropipette
- Caution not to damage the wells with pipetting device.

- The PCR marker was also loaded into one of the wells.
- Performing the electrophoresis.
- The power supply was programmed to give 130 volts and 100 milliamperes for 30 minutes.

Results

This work was carried out at the Microbiology and Immunology Department, Faculty of Medicine, Tanta University and Central Research Laboratory of Ministry of Health during the period from March 2020 to February 2021. This study comprised 100 food samples collected during the period of research from different street vendors, roadside cafeterias, restaurants, open markets and supermarkets in Tanta city. The present study was conducted on 100 food samples. They included meat (10%), chicken (7%), egg (4%), sea food (4%), milk and milk products (7%), cheese (1%), gluten and rice (46%), juice and pastries (14%), vegetables and fruits (7%). **Table 1** showed the association between the main component in the food and Aerobic plate count (APC), there was no statistically significant difference between the main component in the food and colony count. As regard the sanitation status from which samples were collected, overall, 100 samples were examined in this study 12% from PS outlets and 88% from GS outlets. As regard the microbiologic growth among studied samples, microbiologic cultures were done for all studied samples, 73% revealed growth, while 27% revealed no growth. As regard the number of organisms per culture in samples with positive growth and their percentage, out of 73 samples with growth, 56 revealed single growth (76.7%) and 17 mixed growth (23.3%). **Table 2** showed the distribution of different organisms from food samples, culture positive food samples yielded 90 isolates including single growth (56) and mixed growth (17) of two organisms each. The most frequent isolated organisms were Gram negative bacilli (63.3%) followed by Gram positive bacteria (34.5%) and fungi were the least found (2.2%). Among gram negative bacilli, *Klebsiella* was the most frequently isolated bacteria, followed by *Pseudomonas*, *E. coli*,

Salmonella and *Shigella*. Among gram positive bacteria, *Staphylococci* was the most frequently isolated bacteria, followed by *Enterococci* and *Listeria*. All isolated fungi were *Candida* species. **Table 3** showed association of type of organism with food type, *Enterococci* showed higher frequency associated with dairy products (37.5%), followed by carbohydrates (13.3%). *Staphylococci* showed higher frequency associated with carbohydrates (26.7%). *Listeria* showed higher frequency associated with protein (4%), followed by carbohydrates (3.3%). *Klebsiella* showed higher frequency associated with protein (56%), followed by vegetables (42.9%), and dairy product (37.5%). *Pseudomonas* showed higher frequency associated with carbohydrates (16.7%), and vegetables and fruits (14.3%). *Escherichia coli* showed higher frequency associated with protein (16%). *Salmonella* and *Shigella* showed higher frequency associated with protein (8%, 12% respectively) followed by carbohydrates (3.3% for both). There was no statistically significant difference between the type of organism and food type. The result of antibiotic susceptibility tests, we isolated *MRSA*, *MDR pseudomonas*, no isolated *VRE*, and Gram negative bacteria showed resistance to many antibiotics. **Table 4** showed occurrence of *S.aureus* in food products. Where couscous was the most contaminated samples followed masruda, grape leaves and cabbage samples; staphylococci were detected in 45.7% of all examined samples. **Table 5** showed the distribution of enterotoxin gene profiles among the *S. aureus* isolates of the study

From the point of confirmatory molecular detection of enterotoxigenic *S. aureus* isolates identification, out of 16 examined isolates, 6 isolates were proved to be enterotoxigenic. One isolate carried Sec gene, two isolates carried both SEA genes and SEB gene, and three isolates carried SEA, SEB, and SEC genes. None of the isolates contained SED gene. **Figure 1** showed the agarose gel electrophoresis detection of enterotoxin genes, the molecular weight of positive bands of SEA (120bp), SEB (478), SEC (257bp), and SED (317bp).

Table1. The association between the main component in the food and Mean Aerobic plate counts (APC).

Samples N=100					
Food type	Mean±SD (range) (log 10 CFUg -1)	<i>p</i>	Food subtype	Mean±SD (range) (log 10 CFUg -1)	<i>p</i>
Protein (n=25)	7.28±2.5 (4.5-9.2)	0.251	Meat	6.86±2.3 (4.7-9.1)	0.918
			Chicken	7.68±2.2 (6.9-8.2)	0.294
			Egg	6.7±2.2 (4.5-8.9)	0.838
			Sea food	8.23±2.6 (7.4-9.2)	0.116
Dairy product (n=8)	7.15±2.3 (5.5-8.8)	0.687	Milk and milk product	6.96±2.3 (5.5-8.8)	0.943
			cheese	8.1 (8.1-8.1)	0.424
Carbohydrate (n=60)	6.81±2.3 (4.2-8.9)	0.437	Gluten and rice	7.13±2.3 (4.2-8.9)	0.252
			Juice and pastries	5.92±1.6 (4.2-8.2)	0.090
Vegetables and fruits=7	5.97±1.7 (4.2-7.2)	0.261	Vegetables and fruits	5.97±1.7 (4.2-7.2)	0.261

Table 2. The distribution of different organisms from food samples.

Organism	Total isolates n=90	
	N	%
Gram negative bacteria	57	63.3%
<i>Klebsiella</i>	27	30%
<i>Pseudomonas</i>	11	12.2%
<i>E. coli</i>	10	11.1%
<i>Shigella</i>	5	5.6%
<i>Salmonella spp</i>	4	4.4%
Gram positive bacteria	31	34.5%
<i>Enterococci</i>	12	13.3%
<i>Staphylococcus aureus</i>	16	17.9%
<i>Listeria</i>	3	3.3%
Fungus	2	2.2%
<i>Candida</i>	2	2.2%

Table 3. Association of type of organism with food type.

	Protein		Dairy products		Carb		Vegetable		p
	N=25		N=8		n=60		n=7		
	N	%	N	%	N	%	N	%	
<i>Enterococci</i> n=12	1	4%	3	37.5%	8	13.3%	0	0%	0.079
<i>Staphylococcus aureus</i> n=16	0	0%	0	0%	16	26.7%	0	0%	0.646
<i>Listeria</i> n=3	1	4%	0	0%	2	3.3%	0	0%	0.903
<i>Klebsiella</i> n=27	14	56%	3	37.5%	7	11.7%	3	42.9%	0.200
<i>Pseudomonas</i> n=11	0	0%	0	0%	10	16.7%	1	14.3%	0.086
<i>E coli</i> n=10	4	16%	0	0%	6	10%	0	0%	0.615
<i>Salmonella spp</i> n=4	2	8%	0	0%	2	3.3%	0	0%	0.782
<i>Shigella spp</i> n=5	3	12%	0	0%	2	3.3%	0	0%	0.402
<i>Candida</i> n=2	0	0%	1	12.5%	1	1.7%	0	0%	0.339

Fisher exact test was used for comparison.

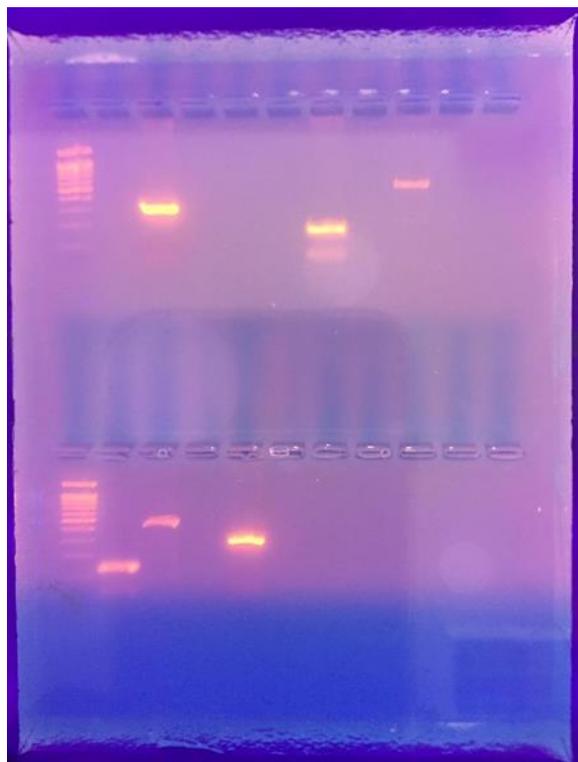
Table 4. Occurrence of *S. aureus* in food products.

Product	Number of analyzed samples	Number of positive samples (%)
Masruda	10	5(50%)
Cabbage	7	2(28.6%)
Couscous	13	6(46.1%)
Grape leaves	5	3(60%)
Total	35	16(45.7%)

Table 5. Distribution of enterotoxin gene profiles among the *S. aureus* isolates.

Origin	No of enterotoxigenic <i>S. aureus</i> isolates	No of enterotoxin gene positive samples			
		Sea	Seb	Sec	Sed
Masruda	1	0	0	1	0
Cabbage	2	1	1	0	0
Couscous	3	1	1	1	0
Grape Leaves	0	0	0	0	0
Total	6	2(33%)	2(33%)	2(33%)	0

Figure 1. Agarose gel electrophoresis detection of enterotoxin genes, the MW of positive bands of sea (120bp), seb(478), sec(257bp),sed(317bp).



Upper lane 1 : control lane , upper lane 3 : positive *Staph.aureus* strain for sec gene , upper lane 7: positive *Staph.aureus* strain for sea gene, upper lane 9: positive *S.aureus* strain for seb gene, lower lane 1 : control lane, lower lane 2: positive *S.aureus* strain for sea gene, lower lane 3: positive *S. aureus* strain for seb gene , lower lane 5: positive *S.aureus* strain for sec gene.

Discussion

In the present study APC was from 4.2 to 9.2 log₁₀ CFUg⁻¹. The highest level observed with protein (7.28±2.5 log₁₀ CFUg⁻¹) followed by Dairy product (7.15±2.3 log₁₀ CFUg⁻¹), then carbohydrate (6.81±2.3 log₁₀ CFUg⁻¹), and last vegetables and fruits (5.97±1.7 log₁₀ CFUg⁻¹), the result of current study more or less similar to other studies in Tanta city [17]. On the other hand, study by Salem et al. [18] recorded the total plate count of Kariesh samples had a mean of 2.14x10⁸CFU/g and a median of 1.35x10⁸ CFU/g. and also Baraheem et al. [19] found a total plate count of 1.1 x10⁹ CFU/g in all Kariesh samples examined . The difference in the Aerobic colony count (ACC) mean values may be attributed to the different areas of food samples sources. It has been noted that plate count of aerobic mesophilic microorganisms found in food is one of the microbiological indicators for

food quality, and most foods are regarded as harmful when they have big count of these microorganisms, even if the organisms are not known to be pathogen [20,21]. According to the present study, approximately (88/100) 88 % of the samples were collected from good sanitation state outlets while (12\100) 12% from bad sanitation state outlets. In the present study, regarding the association of food types with sanitation status, the most frequent type of samples bought from good sanitation outlets was carbohydrates (64.8%) especially gluten and rice (52.5%). The most frequent type of samples bought from bad sanitation outlets was protein (33.3%) especially egg (16.7%), carbohydrates (25%) especially juice and pastries (25%), vegetables and fruits (25%). GS was significantly associated with gluten, rice and carbohydrates ($p=0.001$, 0.012 respectively), while PS was significantly associated with vegetable and fruits, this is in agreement with Bereda et al. [22]. In the present study microbiologic cultures were done for all studied samples, among 100 enrolled samples, microorganisms were isolated from 73% (73/100), while 27% (27/100) revealed no growth, this agreement with, Yusha'u et al. [23]. On the other hand, higher rate of isolation was observed by Asiegbu et al. [24] in the Johannesburg, South Africa who found an isolation rate of 85.37%. The difference can be explained by the informal setting that is usually known for unhygienic operating environment. The presence of microorganisms in ready to eat foods can be considered potentially hazardous to vendors and customers, and hence, these products are not acceptable for consumption. Their presence is attributable to poor hygiene by vendors and unsanitary facilities on the vending site. Therefore, awareness among street consumers through regular trainings on food safety and hygienic practices in food handling is strongly recommended. In the present study, out of 73 samples with growth, 56 revealed single growths (76.7%) and 17 revealed mixed growth (contained two pathogens) (23.3%), culture growth in PS samples was 83.3%, while culture growth in GS samples was 71.6%. No significant association was found between culture results and sanitation status ($p>0.05$). Mixed growth in PS samples was 40%, while mixed growth in GS samples was 20.6%. No significant association was found between number of growth and sanitation status ($p>0.05$), this is in agreement with Heir et al. [25]. According to types of organisms among mixed growth, the most

frequent mixed growth was attributed to *Klebsiella* and *pseudomonas* (29.4%), followed by *E. coli* and *pseudomonas* (17.6%), *E. coli* and *Shigella* (11.8%), *Klebsiella* and *Enterococci* (11.8%), and lastly *E. coli* and *Listeria* (5.9%), *Klebsiella* and *Listeria* (5.9%), *Enterococci* and *candida* (5.9%), *Pseudomonas* and *candida* (5.9%), *Salmonella* spp and *klebsiella* (5.9%), on contrary to our study, **Pal et al.** [26] reported that the mixed growth in the examined cheese sample was attributed to *C. jejuni* and *E. coli* (26.2%), *L. monocytogenes* and *Salmonella* (15.3%), *S. aureus* and *Y. enterocolitica* (11.6%). Hence, the microbiological safety plays a very significant role in the quality of cheese and other dairy products. Additionally, **Das et al.** [27] reported that, milk and dairy products have an outstanding nutritional quality but is also an excellent medium for bacterial growth, the most frequent mixed growth was *Salmonella* and *E. coli* (54%), *Brucella* and *E. coli* (37%), *E. coli* and *L. monocytogenes* (18%). In the current study regarding the distribution of different organisms from food samples, the most frequent isolated organisms were gram negative bacilli (63.3%) followed by gram positive bacteria (34.5%) and fungi were the least found (2.2%). Among gram negative bacilli, *Klebsiella* was the most frequently isolated bacteria, followed by *pseudomonas*, *E. coli*, *Salmonella* and *Shigella*. Among Gram positive bacteria, *Staphylococci* was the most frequently isolated bacteria, followed by *Enterococci* and *Listeria*. All isolated fungi were *Candida* species. The present study revealed that gram-negative bacteria were the commonest isolates 63.3% of isolates, our findings are in accordance with that reported by **Maina et al.** [28]. On contrary to our study, another study in France by **Coton et al.** [29] reported that the most frequently isolated gram-negative bacteria identified in the selected food samples were *Pseudomonas* followed by *Proteus*. Results and the previous studies showed *Pseudomonas aeruginosa* growth in many of the food samples analyzed may be poor hygiene practices of the food handlers, inadequate heating, secondary contamination via contact with contaminated equipment, utensils or surfaces and inappropriate processing. It may also be due to microbial contamination of water used to wash equipment and utensils, large number of people crowding serving space or food sale point and long periods between preparation time and consumption time. *Pseudomonas aeruginosa* can be found in

nearly everywhere as long as there is enough water **Gopal et al.** [30]. Regarding the association of type of organism with food type, *Enterococci* showed higher frequency associated with dairy products (37.5%), followed by carbohydrates (13.3%). *Staphylococci* showed higher frequency associated with carbohydrates (26.7%). *Listeria* showed higher frequency associated with protein (4%), followed by carbohydrates (3.3%). *Klebsiella* showed higher frequency associated with protein (56%), followed by vegetables (42.9%), and dairy product (37.5%). *Pseudomonas* showed higher frequency associated with carbohydrates (16.7%), and vegetables and fruits (14.3%). *Escherichia coli* showed higher frequency associated with protein (16%). *Salmonella* and *Shigella* showed higher frequency associated with protein (8%, 12% respectively) followed by carbohydrates (3.3% for both), in agreement with our study **Amare et al.** [31]. Results of antibiotics susceptibility tests were in agreement with **Marino et al** [32]. Out of 16 identified *S. aureus* strains recovered from the examined samples, 6 isolates were proved to be enterotoxigenic. One isolate carried Sec gene, two isolates carried both SEA genes and SEB gene, and three isolates carried SEA, SEB, and SEC genes. None of the isolates contained SED gene, **Saif et al.** [33] and **Ali and Ab-Elaziz** [34] found a similar result.

Conclusion

Some of food and drink samples collected from different fast food restaurants in Tanta city with different sanitary quality may pose a considerable risk to public health, the sea food were the most frequent samples contaminated with pathogens, we isolated enterotoxigenic MRSA from couscous, masruda, grape leaves and cabbage.

Conflict of Interest: None.

Funding: None.

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Appendix. Primer sequence of the studied genes.

Gene name	Primer Sequence (5' ->3')
SEA(F)	TTGGAAACGGTTAAAACGAA
SEA(R)	GAACCTTCCCATCAAAAACA
SEB(F)	TCGCATCAAACGACAAACG
SEBb(R)	GCGGTACTCTATAAGTGCC
SEC(F)	GACATAAAAGCTAGGAATTT
SEC(R)	AAATCGGATTAACATTATCC
SED(F)	CTAAGTTGGTAATATCTCCT
SED(R)	TAATGCTATATCTTATAGGG

Abd El karim MA, Eshra K, Hegazy E, Zamzam A. Microbiological study of some commonly used foods and drinks in Tanta Al Gharbia Governorate. *Microbes Infect Dis* 2022; 3(4): 1032-1043.