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ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA FROM READY-TO -EAT FAST FOODS IN AL-QUWAYIYAH, KINGDOM OF SAUDI ARABIA

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ABSTRACT

Food-borne pathogens are becoming a globally formidable health problem and perceived as a major health concern in the Kingdom of Saudi Arabia (KSA). Contamination ensued through unclean raw food materials and particles, use of polluted water, unhygienic preparation processes and use of contaminated containers. Herein, the prevalence of food-borne pathogens in ready-to-eat (RTE) fast foods from fifteen different food eateries such as 7 restaurants, 6 cafeterias and 2 two college canteens in Al-Quwayiyah, Riyadh Region of Saudi Arabia was studied. Microbiological analysis of 155 fast food samples which included, Vegetable salad, Falafel, Kibtha and Shawarma. The isolates were detected using biochemical tests and API 20E and slide agglutination test were conducted for *Salmonella* spp. detection. Bacterial growth was found in all food samples tested. Moreover, the test also showed high levels of total aerobic count: vegetable salad 6.34±0.03, falafel 5.79 ± 0.18, kibtha 5.06 ±0.02 and shawarma 3.54 ± 0.13. Organisms isolated include *Salmonella* spp. (15%), *Escherichia coli* (18%**)** and *Staphylococcus aureus* (7%). *Salmonella* is one of the most virulent pathogen implicated in food-borne disease outbreaks. There are numerous transmission routes for Salmonellosis, but the majority of the human infections are derived from consumption of contaminated poultry products. Consistently, *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Heidelberg are the three most frequent serotypes recovered from humans each year. Serologically identified *Salmonella* serotypes from RTE fast food samples were *Salmonella* Typhimurium with 65%, the most predominant one compared to *Salmonella* Enteritidis that was 35%. The bacterial count of vegetable salad, falahfel, kibtha was statistically significant when compared with Shawarma ($p < 0.05$). This result indicated that most of the ready-to-eat food samples examined in the study did not meet any bacteriological quality standard as recommended by The New South Wales (NSW) Food Authority to be <5.0 log10 CFU g−1 and, therefore, it poses potential risks to consumers. Ready- to- eat fast foods must be cooked and served to the consumers with all hygienic measures.

Key words: foodborne pathogen, microbial quality, Ready- to- eat fast foods, Al-Quwayiyah

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INTRODUCTION

Food-borne disease is a progressively perceived complication involving a wide spectrum of illnesses caused by bacterial, viral, parasitic or chemical contamination of food. The World Health Organization (WHO) reported in the Foodborne Disease Burden Epidemiology Reference Group (FERG) that 31 foodborne pathogens caused 600 million cases of illness leading to 420,000 deaths worldwide [1].

The Kingdom of Saudi Arabia has a well-functioning food safety system. The government has established a food and drug authority and the main accountability for food safety and food laws has recently been amended for those who violate the food safety rules. Strict measures have been taken against the violators in the form of heavy fines, cancellation of their licenses and even imprisonment. The Ministry of Health and Ministry of Municipalities and Rural Affairs collaborate for food safety, with surveillance and food-borne disease outbreak investigations performed by the Ministry of Health [2]. Despite these facts, the country needs to enhance and maintain the intersectoral coordination existing between food safety stakeholders and Public health laboratories need to be established/ bolstered in regions away from Riyadh, but under its jurisdiction, including Al-Quwayiyah to support food-borne disease surveillance and outbreak investigation [3].

In today's world the fast food industry has grown rapidly because of busy and hectic life schedules. Ready-to-eat fast food is one of the most liked and preferred quick-bites that includes Shawarma (cooked chicken, french fries, mayonnaise, pita bread), falafel (boiled egg, parsley, mayonnaise, ketchup, hot sauce, pita bread), vegetable salad and kibtha (cooked beef liver, cheese, samouli bread) that are sold in almost all fast food restaurants in Saudi Arabia. Consumption of poultry meat is appreciably popular in Saudi Arabia, especially chicken. Traditionally, lamb, goat and camel meat had been customary in the diets of Saudis and Gulf Cooperation Council (GCC) [4].

Food-borne diseases are a major problem in developing countries because of lack of personal hygiene and understanding of fundamentals of hygiene. As much as 70% of diarrheal diseases in developing countries are believed to be of food-borne origin [5]. There are many incidences of food-borne diseases which have been reported in different cities of Saudi Arabia. Recent studies in Sulyyel, Riyadh, showed that *Salmonella* spp. caused gastroenteritis among people 21 hours after having lunch/dinner at marriage parties [6].

Food-borne pathogens were recently the cause of disease outbreaks reported to result in high morbidity rates in Hail and Abha: 39 cases of *Staphylococcus aureus* and 26 cases of *Salmonella enteritidis,* respectively [6]. Although food-borne diseases do not always result in acute gastroenteritis, food represents an important vehicle for transmission of food-borne diseases. Diarrheal diseases are the commonest manifestation of food poisoning and in some cases, it is highly lethal too.

However, as per the literature survey, there is no study has been conducted on the microbial safety of the foods supplied by local food establishments in Al-Quwayiyah.

Hence, the present study was carried out to assess the microbiological quality of various ready-to-eat fast foods supplied in Al-Quwayiyah, in a bid to throw more light on the inherent microbial hazards associated with such foods.

MATERIALS AND METHODS

Food samples were collected from 15 selected food outlets in Al-Quwayiyah, located 165 kms away towards west of Riyadh. People around here buy food from at least one of these selected out-lets during various times of the day. These sites were chosen because they are very popular among students, workers, shoppers and passers-by. Restaurants (R), cafeterias (C) and two college canteens (CC) are numbered as R1, R2, R3, R4, R5, R6 and R7, C1, C2, C3, C4, C5 and C6 and CC1, CC2, respectively. This study was conducted between October 2017 to February 2018.

Sample Collection

A total of 155 ready-to-eat fast food samples from 15 restaurants were purchased. This study included 4 different foods, Vegetable salad-35, Falafel-25, Kibtha-15 and Shawarma-80 **(Table 1),** all of which are popular foods from food outlets in the study area. Samples were packed separately and kept in ice boxes that were later transferred to the microbiology research laboratory of the University of Shaqra for immediate study.

Bacteriological analysis

Sample Preparation, Culture and Bacterial Count

Samples were processed, studied- and viable bacterial counts were done according to Roland *et al*. [7] with some modifications. Twenty five grams of each sample were homogenized by blending in 225 ml of sterile buffered peptone water. One millilitre of the homogenate was introduced into 9 ml of the buffered peptone water in a test tube, labelled 1:10 (10^{-1}) dilution and serially diluted to five other test tubes labelled 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. The procedure was repeated for each sample and the blender was carefully cleansed and disinfected in between sampling to prevent any cross contamination. One hundred microliters of each of the diluted samples were plated on nutrient agar (Scharlau, Spain). The plates were then incubated aerobically for 24 h at 37 °C. All discrete colonies were counted and expressed in colony forming units per gram (CFU g^{-1}). To improve recovery and detection, the tubes were again incubated aerobically at 37°C for 24 h after which a loop full of enrichment broth was cultured on Salmonella/Shigella (HIMEDIA, INDIA), Mac Conkey (Scharlau, Spain), XLD agar (Oxid, England), Eosin-Methylene Blue (EMB) (HIMEDIA, INDIA), Baird-Parker agar (Oxid, England) and Blood agar (HIMEDIA, INDIA) supplemented with 5% of sheep blood. After that, the plates were once again incubated aerobically for 24–48 h at 37°C for bacterial growth.

Isolation and Biochemical Characterization of the Isolates

Bacterial colonies were analyzed by colony pigmentation and Gram staining characteristics. Pure cultures were obtained by streaking a portion of an isolated colony on nutrient agar and incubated aerobically at 37 °C for 24 h. The isolates were confirmed by catalase and coagulase activity using MASTASTAPH ™ Kit. Isolates were further characterized biochemically using API 20E strips (BioMerieux- France). The

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confirmation of *Salmonella* spp. were analysed according to *Ben Salem et al.* [8] with some modifications. From each sample, 25 g was pre-enriched in 275 ml buffered peptone water (HIMEDIA, INDIA) at 37°C for 24h. Afterwards, 0.1 ml of the pre-enrichment sample was incubated in 9.9 ml of buffered Rappaport Vassiliadis Medium (HIMEDIA) and was incubated at 42ºC for 24h. Another media was used (2ml /20ml) buffered selenite cystine medium (HIMEDIA) was used for next 24 h at 37°C. The enrichment samples were then applied onto Xylose Lysine Deoxycholate agar (Oxid, England) and Salmonella Shigella agar (HIMEDIA) both of which are selective media used for *Salmonella* spp. Innoculated plates were incubated for 24 h at 37°C. Presumptive positive colonies were identified by Gram staining and oxidase reaction. Both Gram-negative and oxidase-negative isolates were further confirmed with API according to manufacturer's instructions, for presence of *Salmonella* spp. [9]. Serotyping of *Salmonella* strains was performed by using BIO-RAD Kit according to Kauffman-White scheme [10,11] mentioned in table 2.

Statistical Analysis

Statistical Analysis was performed using SPSS version 21(IBM, USA). The bacterial counts were expressed as mean $\pm$ standard deviation. One-way ANOVA followed by Tukey *post hoc test* was used to compare the bacterial counts in various RTE fast food types. Differences in mean bacterial counts between different food types were considered significant when $P<0.05$.

RESULTS AND DISCUSSION

Study of ready to eat fast food (RTE) samples

It was surprising to find the presence of bacteria in RTE food based on the study conducted on samples collected from 15 select outlets. The mean bacterial counts in the RTE fast food samples were expressed as colony-forming unit per gram ($\log_{10}$ CFU g^{-1}). Foods were classified as acceptable if the bacterial counts were less than or equal to 5 $\log_{10}$ CFU g^{-1}. The mean value of aerobic bacterial counts Vegetable salad, Falafel, Kibtha and Shawarma were 6.34 ± 0.03, 5.79 ± 0.18, 5.06 ± 0.02 and 3.54 ± 0.13 $\log_{10}$ CFU g^{-1} respectively (table 3). The bacterial count of vegetable salad, falahfel, kibtha was statistically significant when compared with Shawarma ($p < 0.05$).

The contamination levels in this study of all the samples had mean bacterial counts ≥ 5.0 $\log^{10}$ CFU g^{-1}. The New South Wales (NSW) Food Authority recommends the standard limit for bacterial count of fully cooked ready-to-eat foods to be <5.0 $\log_{10}$ CFU g^{-1} [12]. Hence, most of the samples in this study are not of good quality according to NSW standard. These findings authenticate previous works [13]. In their study [14], high bacterial count was found in salads sold on the streets of Accra. Likewise, [15] the study also reported high bacterial prevalence in filled baguettes and salads.

Vegetable salad had the highest bacterial count of 5.74-6.8 log10 CFU g^{-1}. The findings are in agreement with other studies in Alice, South Africa. Roland *et al.* [7] reported a similar upper limit of 6.8 log10 CFU g^{-1} aerobic counts of microorganisms on minimally processed vegetable samples in Egypt while in Taiwan, an aerobic plate count of 8.64

log10 CFU g^{-1} on salad vegetable samples was reported [16]. Furthermore, an almost similar range of aerobic plate counts was reported in Tabuk, Saudi Arabia [17].

This study finds that RTE fast food samples taken from Al-Quwayiyah had significant growth of microorganisms, but the bacterial count in RTE fast food samples obtained from Vegetable salad and Falafel were higher compared to Kibtha and Shawarma and this is similar to study conducted by Andino *et al.* [18] as they isolated most similar bacteria from Shawarma, Falafel and Vegetable salad.

Types of Foodborne pathogens in the RTE fast food collected

Table 4 depicts the occurrence of possible pathogens in the 155 food samples tested from 15 select outlets. Bacterial growths were observed in all the RTE fast food types. The most prevalent bacteria were *Salmonella* spp. (15%), *E. coli* (18%) and *S. aureus* (7%). *Salmonella* spp. was found in kibtha (27%), Vegetable salad (23%), Falafel (12%) and Shawarma (10%). *E. coli* isolated from Falafel was 36%, Kibtha 33%, Vegetable salad 17% and Shawarma (10%). *S. aureus* detected in Kibtha was 13%, 5% in Shawarma and 8% each in Vegetable salad and falafel.

Enteropathogenic E.coli is a normal flora of the human and animal intestine and has been identified as a leading cause of foodborne illness all over the world [19]. Moreover, diarrhea caused by *E.coli* is highly prevalent in young children in developing countries and as well as among travelers. However, *E.coli* 0157.H7 strain was not detected in any of the collected RTE fast food samples. The prevalence of indicator and other organisms examined in this study is of special concern. These organisms are perhaps a threat associated with usage of water contaminated with human excreta [20] for processing food for human consumption.

S. aureus was prevalent in 4% of the samples. The results were contrary to the findings of Ghosh *et al*., [21] who reported that *S.aureus* was detected in 86% samples of ready-to-eat salads in India. Toxins produced by *S. aureus* are one of the most frequent causes of bacterial food poisoning. The pathogenic nature of *S. aureus* is related to high genotypic and phenotypic heterogeneity of its strains. In this study, *S. aureus* isolated from RTE fast food samples was 5% in Shawarma, 13% in Kibtha. These were lower than the reported counts in the Egypt [22]. *S.aureus* was isolated from 4 (5%) of the sandwiches, mainly from the chicken sandwiches (4 out of 80). Compared to this study, higher contamination rates with *S. aureus* were reported in Sudan [23].

In this study, a series of experiments were conducted for isolation of *Salmonella* species from RTE fast food samples. Out of all 155 samples tested, only 15% was found to be contaminated with *Salmonella*. Nowadays, *Salmonella* is one of the most virulent genera implicated in food-borne bacterial outbreaks and diseases and constitutes an important public health problem. There are numerous transmission routes for Salmonellosis, but the majority of the human infections are derived from consumption of contaminated poultry products [24]. Table 5, clearly showed that, of the serologically identified *Salmonella* serotypes from RTE fast food samples, *Salmonella* Typhimurium (65%) was most predominant compared with *Salmonella* Entritidis (35%). Consistently, *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Heidelberg are the three most

frequent serotypes recovered from humans each year [25]. These data show a lower incidence than that found in previous studies in Northern Greece and South Africa, where 17.5% and 20%, respectively of the sandwiches tested were found to be contaminated with *Salmonella* spp. The specific salad consisted of raw vegetables, chicken and mayonnaise-base dressing. *Salmonella* spp. was detected by previous researchers in Spain, South Africa, Turkey, Nigeria with 0.7%, 11%, 8.0% and 8.0% incidence, respectively [26].

CONCLUSION

The findings in the study conducted on collected food samples from fifteen food outlets revealed that the ready-to-eat fast food (RTE) sold at Al-Quwayiyah, region of Riyadh, Kingdom of Saudi Arabia were found to be contaminated with pathogenic bacteria such as *Salmonella* Spp., *E.coli, S.aureus*. This contamination could be due to unhygienic handling of food and use of stale vegetables in RTE which might be cross contaminated with various pathogens. Some of these diseases could spread and acquire epidemic status which poses serious health hazards. Ready-to-eat fast food cooked and served to the consumers should be safe for human consumption and prepared with all hygienic measures. Hence, it is recommended that all fast food outlets must follow the food safety standards and microbial quality control rules of KSA in Al-Quwayiyah region.

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Conflict of interest statement
We declare that we have no conflict of interest.

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Table 1: Samples of RTE Fast Food from Fifteen Food Outlets

Sample collection	RTE fast food samples and Ingredients			
	Shawarma (cooked chicken, french fries, mayonnaise, pita bread)	**Falafel** (boiled egg, parsley, mayonnaise, ketchup, hot sauce, pita bread)	**Vegetable salad** (parsley, cucumber, carrot, tomatoes, onion)	**Kibtha** (cooked beef liver, cheese, samouli bread)
R1	7	2	4	2
R2	6	2	4	2
R3	6	2	3	1
R4	8	2	3	1
R5	3	1	3	1
R6	3	1	3	1
R7	4	1	3	1
C1	5	1	2	1
C2	5	1	2	1
C3	7	1	2	1
C4	6	2	2	1
C5	5	2	2	1
C6	5	2	2	1
CC1	5	3	-	-
CC2	5	2	-	-
Total Sample =155	**80**	**25**	**35**	**15**

RTE; ready to eat , R; Restaurants, C; Cafeterias, CC; College Canteen

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Table 2: Identification of *Salmonella enterica* subsp. *enterica* serotypes based in RTE Samples

Serotype	O Antigens	H Antigens Phase 1	H Antigens Phase 2
Enteritidis	1,9,12	[f],g,m,[p]	[1,7]
Typhimurium	1,4,[5],12	i	1,2

Table 3: Presence of Mean bacterial counts of the RTE fast food samples examined

RTE food types	Bacterial count range ($\log_{10}$ CFU g^{-1})	Mean bacterial count ($\log_{10}$ CFU g^{-1}) ± SD	*p-value*			
			V	F	K	S
Vegetable salad (n=35)	5.74-6.8	6.34 ± 0.03	-	0.365	0.613	0.000
Falafel (n=25)	5.28-6.1	5.79 ± 0.18	0.365	-	0.001	0.000
Kibtha (n=15)	4.76-5.61	5.06 ±0.02	0.613	0.085	-	0.000
Shawarma (n=80)	3.10-4.28	3.54 ± 0.13	0.000	0.000	0.000	-

CFU g^{-1}, colony forming units per gram; SD, standard deviation; no comparison done. The mean difference is considered significant at $p < 0.05$

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Table 4: Bacteria distribution in the various RTE fast food samples

Bacteria isolates	Shawarma (n=80)	Falafel (n=25)	Vegetable salad (n=35)	Kibtha (n=15)	Number (%) 0ccurence
Salmonella spp.	8	3	8	4	23/155 (15%)
E. coli	8	9	6	5	28/155 (18%)
Staphylococcus aureus	4	2	3	2	11/155 (7%)
Total isolates	**20**	**14**	**17**	**11**	**62**

RTE; ready to eat, *E.coli*; Escherichia coli, n; number, %; Percentage

Table 5: Confirmation of *Salmonella* Serovars using Serological Technique

Identified Serovars of *S. entrica*	RTE fast food samples				Total	
	Shawarma	**Falafel**	**Vegetable salad**	**Beef liver**		
	No	**No**	**No**	**No**	**No**	**%**
Salmonella entrica serovars Typhimurium	5	2	6	2	15	65
Salmonella entrica serovar Enteritidis	3	1	2	2	8	35
Total	**8**	**3**	**8**	**4**	**23**	**100**

RTE; ready to eat, No; Number, %; Percentage

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