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Risk of foodborne pathogens in various food products at retail in Qatar

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ABSTRACT

Foodborne illness is a major limitation to the advancement of world health. Bacterial pathogens among the leading causes of foodborne illness include *Escherichia coli*, *Campylobacter*, *Salmonella*, and *Listeria*. In an effort to understand the risk these foodborne pathogens pose regionally, this study investigated the presence of these pathogens among retail products in Qatar. Using a combination of bacterial selection and molecular detection, swabs and food samples collected from retail items (n = 287) were screened for the presence of these foodborne pathogens. E. coli O157:H7 was detected in 4.2% of all samples tested. Other *E. coli* serogroups were detected at varying proportions across all samples: O26 (5.9%), O111 (3.5%), O121 (1.4%), O45 (20.2%), O103 (2.1%) and O145 (2.1%). The occurrence of the other pathogens varied: *Salmonella* (13.6%), *Listeria* (5.2%), *C. jejuni* (1%), *C. coli* (8%) and *C. lari* (0%). While *E. coli* O157:H7 arguably receives the most attention in STEC surveillance programs, our study shows that other food adulterant serotypes, such as *E. coli* O45, could potentially play a role in infection. Although the prevalence for many pathogens is low, the higher occurrence of STEC genes and STEC serotype O45 is a reason for concern.

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1. Introduction

Foodborne illness is a major health burden worldwide. The Centers for Disease Control and Prevention (CDC) estimate that 48 million people become ill due to foodborne diseases each year in the US alone, 128,000 infections result in hospitalization with 3,000 infections resulting in death (1). The World Health Organization (WHO) estimates that 2.2 million people per year worldwide die of diarrheal food and waterborne diseases (2) and the Foodborne Diseases Burden Epidemiology Reference Group (FERG) with WHO is currently conducting an estimation of the worldwide burden of foodborne disease. However, current estimations by individual nations indicate that, in the aggregate, foodborne illness is a costly public health challenge across nations (3,4).

The risk of foodborne pathogen transmission is increased by the expansion of travel and the trade globalization, including food products (5). This is especially true in internationalized areas such as Qatar, where the ratio of expatriates to natives is 9:1 (https://www.mdps.gov.qa/en/statistics1/StatisticsSite/Pages/Population.aspx). Areas of such cultural diversity may offer unique opportunities to study different facets of foodborne illness, especially the impact of import markets.

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Campylobacter and *Salmonella* are two major foodborne pathogens responsible for foodborne illness, especially in much internationalized regions (6). Consumers are more likely to contract foodborne illnesses by food consumption in public places due to potential post retail contamination. Accurate, region-specific surveillance data on the transmission of foodborne pathogens, however, is required to mitigate the risks of transmission.

Retail food products can become contaminated by numerous means and through numerous channels. Recontamination is a major concern in factories, stores, restaurants and in the home (7,8,9). Several studies have investigated the capacity of different foodborne pathogens to survive on different surfaces found in food processing environments, such as stainless steel, aprons, gloves and hands (10,11). Confounding matters, some foodborne pathogens can form biofilms, making the bacterial community more impervious to standard cleaning methods (7). Several groups have developed models in an effort to estimate food safety risks associated with different products or production practices, but with varying results (12,13). Our objective was to assess the prevalence of major foodborne pathogens among retail food samples available to a highly dynamic and diverse population to shed further light on the food safety risks associated with different types of retail food in Qatar.

2. Materials and methods

2.1. Sample collection

This study and its design were approved by the Municipality of Doha. Municipal officers recommended sampling from five major retail stores and five large restaurants and accompanied our research team on sampling trips. At the retail stores, samples were taken from different types of meat, packages, displays and processing areas. At restaurants, samples were taken from the processing area before storage, the storage area and the food preparation area.

Sterile gauze pads (4x4in) were used to swab the surfaces and utensils using aseptic technique and the swab samples were immediately placed into a sterile vial. Cuts of meat samples, cheese, and samples of ready-to-eat (RTE) food were collected aseptically and placed directly into the sterile tubes. Various types of foods were sampled, including beef, chicken, lamb, goat, camel, seafood (fish, shrimp, crab, cuttlefish, and squid), cheese and salads. Ready to eat (RTE) foods included deli meats, cheeses and salads. Surfaces included tables, cutting boards, knives, containers, refrigerators, serving plates, gloves, and balances and mincing machines. Sample sizes are detailed in Table 1. All samples were transported to Weill Cornell Medical College of Qatar in ice boxes for processing.

2.2. Sample Processing and Pathogen Detection

Samples were screened for *E. coli O157:H7*, non-O157 shiga toxin-producing *E. coli*, *Salmonella*, *C. jejuni*, *C. coli*, *C. lari*, and *L. monocytogenes* using the BAX® System (Hygiena, USA). The BAX® system utilizes a real-time polymerase chain reaction (PCR). Prior to PCR, samples undergo different enrichment steps depending on the targeted bacteria as described below.

2.2.1. *E. coli O157:H7* and *STEC*

To detect *E. coli O157:H7*, samples were first enriched in 100 mL Modified *E. coli* broth (MEC broth [Oxoid, ThermoFisher Scientific; Waltham, MA, USA]) containing novobiocin at 8 mg/L) at a ratio of 1:10. The inoculum was incubated for 24hr at 37°C. A total of 20 µL of the resulting culture was transferred into 1 mL of the secondary enrichment medium (Brain Heart Infusion [BHI] without antibiotics) and incubated for three hours at 37°C before analysis by real-time PCR as described below.

Non-O157 Shiga Toxin-producing *E. coli* were detected by first preparing samples as described for *E. coli O157:H7*. The twice enriched samples were initially screened using the BAX® STEC Suite kit which targets the stx1, stx2 and eae genes. Positive samples were then serotyped based on O antigens O26, O45, O103, O 111, O121, and O145 with additional BAX® STEC kits.

2.2.2. *Salmonella spp.*

Salmonella was detected by first enriching samples in 100 mL of Tetrathionate broth (Becton, Dickinson and Company; Sparks, MD, USA). Samples were incubated at 42oC for 20-24 hr. Enriched samples were then spread on XLT4 agar plates (MOLTOX®, Boone, NC, USA). Positive colonies (black in color, non-acid forming) on XLT4 were then transferred to 500 µL of BHI broth (secondary enrichment) and incubated for 24hr at 37°C prior to real-time PCR assay.

2.2.3. *Campylobacter jejuni, coli* and *lari*

Campylobacter was detected by first enriching samples in 100 mL BHI supplemented with

cefoperazone (6 mg/L), vancomycin (6 mg/L), and amphotericin B (2 mg/L). Samples were incubated at 37oC for 24 hr. The secondary enrichment also was 1 mL BHI and was also incubated at 37°C for 24 hr.

2.2.4. *L. monocytogenes*

L. monocytogenes was detected by first pre-enriching samples in 100 mL Demi-Fraser broth (Oxoid) and incubating for 22-26hr at 30°C. Pre-enriched samples (20 µL) were then transferred to 1 mL MOPS Buffered Listeria Enrichment Broth (Fisher Scientific) and samples were again incubated at 35°C for 18-24hr prior to real-time PCR assay.

2.2.5. PCR Detection

The PCR detection was performed using the BAX® Automated System according to the directions of the manufacturer. Briefly, a 5µL aliquot of each final culture was added to 200 µL of lysis buffer (proteinase-containing lysis) provided by the manufacturer. Samples were then heated in a lysis reagent solution to rupture the bacterial cell wall and release nucleic acid. Lysed samples were added to PCR tablets, containing all the PCR reagents including a fluorescent dye. More details about the system are available at https://www.hygiena.com/bax.html. Briefly, the tablets were hydrated with a lysed sample and processed in the cycler/detector provided by the manufacturer. Within a few hours, the polymerase chain reaction (PCR) amplified a DNA fragment specific to the target. The amplified DNA generates a fluorescent signal, which the BAX® system application uses to analyze the findings.

2.3. Statistical Analysis

The prevalence of a particular pathogen or serogroup was computed as the percentage of the samples testing positive out of all samples from a particular food. The odds of a particular pathogen within each category was evaluated using logistic regression and quantified using the odds ratio (OR). All statistical tests were performed using SPSS v.23 (IBM-statistical Software, White Plains, NY). Differences were considered statistically significant at $P < 0.05$.

3. Results

Across all sample groups, 4.2% of all samples (n = 287) tested positive for the presence of *E. coli O157:H7*. *E. coli* genes stx and eae, which are linked to Shiga toxin-producing *E. coli* (STEC), were detected in 26.8% and 26.5%, respectively, of all samples. Samples containing both stx and eae genes (16.7% of all samples) were considered positive for STEC (Figure 1).

Samples were also tested for the presence of other common non-O157 STEC that are known as food adulterants. Of all samples tested, 5.9% were positive for *E. coli O26*, 3.5% for O111, 1.4% for O121, 20.2% for O45, 2.1% for O103 and 2.1% for O145 (Figure 1). *Salmonella spp.* were detected in 13.6% of all samples and *L. monocytogenes* was detected in 5.2 % of all samples. None of the samples were positive for *C. lari*, but 1% were positive for *C. jejuni* and 8% were positive for *C. coli* (Figure 1).

Table 1 shows the distribution of *E. coli* serogroups by food type. *E. coli O157:H7* was most frequently isolated from beef samples (beef chops and minced beef), and was not detected in chicken samples. None

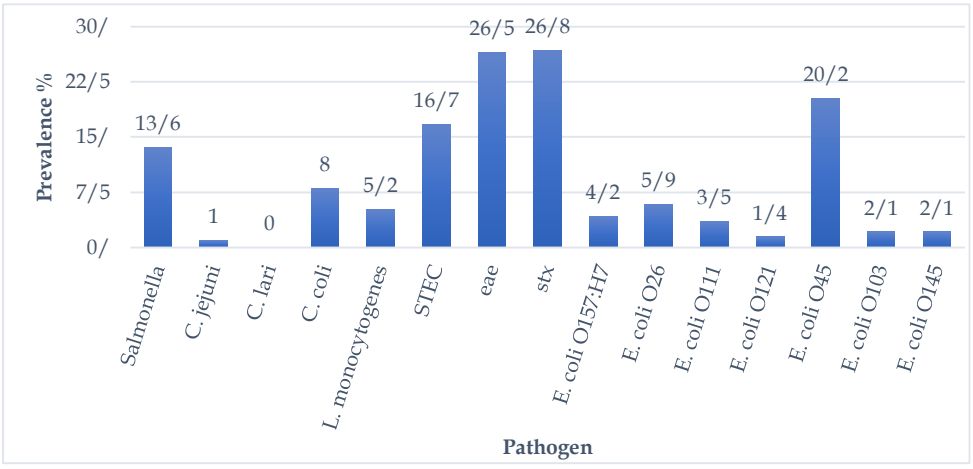

Figure 1. Overall prevalence of the targeted foodborne pathogens among the samples

Table 1. The occurrence of *E. coli O157:H7* and the other non-O157 detection among the different retail products and surfaces surveyed in the study.

Food type	*E. coli* serogroup						
	O157:H7	**026**	**045**	**0103**	**0111**	**0121**	**0145**
Beef [40][a]	4 (10%)[b]	4 (10%)	7 (17.5%)	1 (2.5%)	0	2 (5.0%)	1 (2.5%)
Chicken [21]	0	2 (9.5%)	7 (33.3%)	3 (14.3%)	2 (9.5%)	0	0
Mutton [30]	2 (6.7%)	4 (13.3%)	4 (13.3%)	0	0	0	2 (6.7%)
Seafood [62]	1 (1.6%)	0	14 (22.6%)	0	4 (6.5%)	0	1 (1.6%)
Cheese [11]	0	0	0	0	0	0	0
RTE [55]	3 (5.5%)	3 (5.5%)	13 (23.6%)	1 (1.8%)	3 (5.5%)	2 (3.6%)	1 (1.8%)
Surface [68]	2 (2.9%)	4 (5.9%)	13 (19.1%)	1 (1.5%)	1 (1.5%)	0	1 (1.5%)
Total [287]	12 (4.2%)	17 (5.9%)	58 (20.2%)	6 (2.1%)	10 (3.5%)	4 (1.4%)	6 (2.1%)

[a]: Number of samples tested

[b]: Number (percentage) of samples testing positive

Table 2. The occurrence of *Salmonella spp., L. monocytogenes, C. jejuni,* and *C. coli* detection among the different retail products and surfaces

Food type	*Salmonella spp*	*L. monocytogenes*	*C. jejuni*	*C. coli*
Beef [40][a]	8 (20%)[b]	5 (12.5%)	0	0
Chicken [21]	5 (23.8%)	0	1 (4.8%)	1 (4.8%)
Mutton [30]	6 (20%)	2 (6.7%)	0	3 (10%)
Seafood [62]	0	4 (6.5%)	1 (1.6%)	6 (9.7%)
Cheese [11]	0	0	0	1 (9.1%)
RTE [55]	5 (9.1%)	3 (5.5%)	1 (1.8%)	5 (9.1%)
Surface [68]	15 (22.1%)	1 (1.5%)	0	7 (10.3%)
Total [287]	39 (13.6%)	15 (5.2%)	3 (1.0%)	23 (8%)

[a]: Number of samples tested

[b]: Number (percentage) of samples testing positive

of the *E. coli* serogroups were detected in cheese samples. Serogroup O45 had the highest occurrence, especially among seafood, RTE, and chicken samples. The seafood and chicken samples were fresh samples, while the RTE samples consisted of seasoned chicken, seasoned beef, and chicken cooked with rice. The serogroups O103, O111, O121, and O145 were not common among the samples (Table 1).

The detection of *Salmonella spp., L. monocytogenes, C. jejuni,* and *C. coli* by type of food is shown in Table 2. *Salmonella spp.* was detected from beef, chicken, mutton, and surface samples. There was no significant difference in the odds of detection of *Salmonella spp.* from most sample types when compared to beef, but it was 2.5 (inverse of odds ratio) times greater to isolate it from beef compared to RTE food (Table 3). *L. monocytogenes* was detected in beef, mutton, seafood, RTE, and surfaces, but there was no significant difference in the probability of detection of *L. monocytogenes* across different food types (Table 2). *C. jejuni* was detected in chicken, seafood, and RTE samples at a low probability. However, *C. coli* was detected at a higher proportion in mutton, seafood, cheese, RTE, and surface samples compared to other samples (Table 2).

Table 3 shows the odds of detection of the STEC and *Salmonella* among the different food types. There was a significant association between the odds of these groups and the food type. Beef samples had the highest odds of harboring STEC pathogens compared to other food types ($P < 0.05$). The odds were between 12 and 14 times greater than chicken and seafood samples, more than two times greater than surfaces, and three times greater compared than RTE foods (inverse of odds ratios, Table 3).

We then combined the samples into four categories of fresh meat (beef, mutton, chicken), RTE (RTE, cheese), seafood, and surface samples to further evaluation of the odds of these pathogens (Table 4). The odds were 3.2 (inverse of odds ratio) times greater to detect *Salmonella spp.* in meat samples than in RTE. There were no significant differences, however, in the detection of *Salmonella* between meat and other types of samples. It appeared that the odds of detecting *Campylobacter spp.* were about two times more in other samples compared to meat; however, these differences were not statistically significant (Table 4). The odds of detecting STEC in RTE and seafood samples was less than in meat samples, but again these differences were not statistically significant (inverse of the OR = 2.6, 11, respectively; Table 4).

4. Discussion

There are few studies examining the prevalence of various foodborne pathogens in retail food products in Qatar. Our results differed somewhat from those of

Table 3. The likelihood of STEC and *Salmonella spp*. among the different retail products and surfaces

Food type	STEC			*Salmonella*		
	Regression coefficient	Standard error	Odds ratio and 95% CI	Regression coefficient	Standard error	Odds ratio and 95% CI
Beef [40][a]	0		1.0	0		1.0
Chicken [21]	-2.46	1.08	0.08 (0.01, 0.7)	0.22	0.65	1.3 (0.4, 4.4)
Mutton [30]	-0.50	0.53	0.6 (0.2, 1.7)	0	0.60	1.0
Seafood [62]	-2.89	0.79	0.07 (0.01, 0.3)	NS		1.0
Cheese [11]	NS		1.0	NS		1.0
RTE [55]	-1.26	0.50	0.3 (0.1, 0.8)	-0.92	0.61	0.4 (0.1, 1.3)
Surface [68]	-0.84	0.44	0.4 (0.2, 1.0)	0.12	0.49	1.1 (0.4, 3.0)
Total [287]	-0.51	0.33		-1.39	0.40	

Table 4. The likelihood of the foodborne pathogens among meat, RTE, seafood and surface categories.

Category	Regression coefficient	Standard error	p-value	Odds ratio	95% CI
			Salmonella		
Meat	0		0	1	
RTE	-1.17	0.53	0.03	0.31	(0.11, 0.88)
Seafood	-19.87	5104.51	1	0	0
Surface	0.07	0.39	0.86	1.07	(0.5, 2.30)
Constant	-1.33	0.26	0	0.26	
			Campylobacter		
Meat	0		0.57	1	
RTE	0.71	0.61	0.24	2.04	(0.62, 6.74)
Seafood	0.78	0.61	0.20	2.19	(0.66, 7.24)
Surface	0.68	0.61	0.26	1.97	(0.60, 6.51)
Constant	-2.85	0.46	0	0.06	
			STEC		
Meat	0		0.006	1	
RTE	-0.95	0.45	0.03	0.39	(0.34, 1.53)
Seafood	-2.38	0.76	0.002	0.09	(0.16, 0.92)
Surface	-0.32	0.38	0.40	0.72	(0.02, 0.41)
Constant	-1.03	0.24	0	0.36	

similar studies in that other studies showed a higher prevalence of *Listeria, Campylobacter* and *Salmonella*. In a Canadian study on raw poultry and meat products, only one beef sample was positive for STEC, but 30% of raw chicken legs were positive for *Salmonella*, while 52% of raw ground beef and 34% of raw chicken legs were positive for *L. monocytogenes* (14). In a study of retail meats in the Washington D.C. area, 70.7% of chicken samples were positive for *Campylobacter*, 38.7% were positive for *E. coli* and 9% were positive for *Salmonella*. In the same study, only one beef sample was positive for *Campylobacter*, but 21.7% of samples were positive for *E. coli* and 3% of samples were positive for *Salmonella* (15). In a study on the prevalence of pathogens in retail food in Japan, including raw meats, fruits, vegetables and seafood, *Salmonella* was detected in 33.5% of ground chicken samples and 12.7% of raw (solid cuts) chicken samples. *E. coli* was found in 57.5% of ground beef samples, 75.6% of ground chicken samples, 18.2% of raw beef samples, and 22.8% of raw chicken samples. *C. jejuni* and *C. coli* were found in 20.9% of ground chicken and 13.3% of raw chicken samples (16). None of the studies specifically tested for different serotypes of *E. coli* other than O157:H7 or general STEC genes. The observed non-significance could be attributed to be the low sample size, different methodologies for detection, different region, or different sample types.

Many species of bacteria including *Salmonella* and *E. coli* can survive on surfaces for extended periods of time, in some cases for weeks. This increases the risk of food becoming contaminated if proper cleaning and sterilization methods are not utilized. Bacteria can survive on many surfaces, including hands, utensils, aprons, sponges and even stainless steel surfaces (9,11). Storage areas such as refrigerators are also of concern because some bacteria, like *L. monocytogenes*, can replicate at low temperatures, albeit at lower rates (10). One possibility for our higher rate of STEC is that minced beef is a very popular dish so there could be an increased chance for cross-contamination from raw beef on surfaces.

An estimated 25% foodborne illness outbreaks are due to recontamination (8). Recontamination can be

caused by either direct or indirect contact with surfaces, environmental vectors or air. Ready-to-eat meals are often contaminated by manual contact, poultry and dairy products by surface contact, and ice cream and powders by air. In food processing facilities, biofilms on non-food contact surfaces are additional cause for concern as they are often resistant to disinfection and can be mechanically spread to other surfaces and food itself via aerosolization during cleaning (7). Among processed foods, recontamination is usually caused by insufficient hygiene, improper storage and contaminated equipment or personnel. Soiled packaging is also the main cause (8).

It is clear that eliminating or limiting foodborne pathogen contamination and transmission requires a multifaceted approach. Public perception and practices, however, must also be taken into account, especially in the introduction of new technologies such as irradiation. Finally, there is a responsibility to the consumer. Food products, especially animal products, should not be treated as sterile. Thus, new technologies should also be coupled with educational or outreach programs focused on proper storage, preparation or different food to minimize foodborne illness (12).

5. Conclusions

While *E. coli O157:H7* arguably receives the most attention in STEC surveillance programs, our study shows that other food adulterant serotypes, such as *E. coli O45*, could potentially be playing a role in infection. Although the prevalence for many pathogens is low, the higher occurrence of STEC genes and STEC serotype O45 is cause for concern. Although the prevalence of many pathogens in our sample set was comparatively low, the higher prevalence of STEC genes and STEC serotype O45 is cause for concern. Future efforts may include these as targets in developing mitigation strategies. These recommendations are more applicable to Qatar based on the findings of this paper, but with modifications would be applicable in other regions based on their findings.

Conflict of interest

The authors have no conflict of interest.

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References

1. Centers for Disease Control and Prevention. 2015. Estimates of foodborne disease in the United States. Available at: http://www.cdc.gov/foodborneburden/. Cited: May 2017.
2. World Health Organization. 2015. Food safety: Global burden of foodborne diseases. Available at: http://www.who.int/foodsafety/areas_work/foodborne-diseases. Cited: May 2017
3. Havelaar AH, Haagsma JA, Mangen MJ, et al. Disease burden of foodborne pathogens in the Netherlands, 2009. Int J Food Microbiol. 2012; 156: 231-238.
4. Hoffmann S, Batz MB, Morris JG, Jr. Annual cost of illness and quality-adjusted life year losses in the United States due to 14 foodborne pathogens. J Food Prot. 2012; 75: 1292-1302.
5. Käferstein FK, Motarjemi Y, Bettcher DW. Foodborne disease control: a transnational challenge. Emerg Infect Disease 1997; 3: 503-510.
6. Hird S, Stein C, Kuchenmuller T, et al. Meeting report: Second annual meeting of the World Health Organization initiative to estimate the global burden of foodborne diseases. Int J Food Microbiol 2009; 133: 210-212.
7. Den Aantrekker ED, Boom RM, Zwietering MH, et al. Quantifying recontamination through factory environments - a review. Int J Food Microbiol 2003; 80: 117-130.
8. Reij MW, den Aantrekker ED, ILSI Europe Risk Analysis in Microbiology Task Force. Recontamination as a source of pathogens in processed foods. Int J of Food Microbiol. 2004; 91: 1-11.
9. Kusumaningrum HD, Riboldi G, Hazeleger WC, et al. Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. Int J Food Microbiol 2003; 85: 227-236.
10. Jackson V, Blair IS, McDowell DA, et al. The incidence of significant foodborne pathogens in domestic refrigerators. Food Control. 2007; 18: 346-351.
11. Lues JFR, Van Tonder I. The occurrence of indicator bacteria on hands and aprons of food handlers in the delicatessen sections of a retail group. Food Control 2007; 18: 326-332.
12. Havelaar AH, Brul S, de Jong A, et al. Future challenges to microbial food safety. Int J Food Microbiol 2010; 139; S79-S94.
13. Newell DG, Koopmans M, Verhoef L, et al. Food-borne diseases-The challenges of 20 years ago still persist while new ones continue to emerge. Int J Food Microbiol 2010; 139: S3-S15.
14. Bohaychuk VM, Gensler GE, King RK, et al. Occurrence of pathogens in raw and ready-to-eat meat and poultry products collected from retail marketplace in Edmonton, Alberta, Canada. J Food Prot 2006; 69: 2176-2182.
15. Zhao C, Ge B, de Villena J, et al. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella*

serovars in retail chicken, turkey, pork and beef from the greater Washington, D.C., area. Appl Environ Microbiol 2001; 67: 5431-5436.
16. Hara-Kudo Y, Konuma H, Kamata Y, et al. Prevalence of the main food-borne pathogens in retail food under the national food surveillance system in Japan. Food Addit. Contam. Part A 2013; 30: 1450-1458.