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Detection of Verotoxic *Escherichia coli* in Street Vended Grilled Meat

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Verotoxin producing *Escherichia coli* (VTEC), particularly O157:H7 serotype is more significant than other well-recognized food-borne pathogens and its contamination is known to be largely associated with raw and undercooked meat. This study was aimed to detect VTEC in street vended grilled meats. A total of 75 grilled meat samples (25 samples each for chicken, pork and mutton) were collected from street vendors in Latha Township and tested for coliforms, fecal coliforms and *Escherichia coli* by standard microbiological analysis procedures and verotoxin production was detected by Reverse Passive Latex Agglutination (RPLA) test. Out of 75 samples tested, coliforms were isolated from 58 samples (77.33%), fecal coliforms in 45 samples (60%) and *Escherichia coli* in 29 samples (38.67%). Coliform, fecal coliform and *Escherichia coli* counts were ranging from 3 to >1,100 MPN/g. Verotoxin 1 (VT1) was detected in 3 isolates (1 isolate from grilled chicken and 2 isolates from grilled pork), Verotoxin 2 (VT2) in 3 isolates (2 isolates from grilled chicken and 1 isolate from grilled pork) and both VT1 and VT2 in 3 isolates (all from grilled pork). Among the isolated *Escherichia coli*, 2 isolates were found to be of O157K+ serotype (1 from grilled pork and 1 from grilled chicken). As VTEC can infect even with as low as 10 organisms and causes severe complications like Hemorrhagic Colitis and Hemolytic Uremic Syndrome, the presence of VTEC strains in the tested samples is of considerable risk to the health of consumers and highlights the importance of food safety interventions.

INTRODUCTION

Escherichia coli (*E. coli*) was established as a food-borne pathogen in 1971 when imported cheese contaminated with an enteroinvasive strain of serogroup O124 caused illnesses in nearly 400 individuals in 14 American states.[1] Pathogenic *E. coli* are classified into 6 categories by means of their virulence features: Enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), entero-invasive *E. coli* (EIEC), enteroagreegative *E. coli* (EaggEC), and diffusely adhesive *E. coli* (DAEC).[2] Among pathogenic *E. coli* that cause food-borne illnesses, EHEC are more significant than other *E. coli* in recent years because public health problems with EHEC are being recognized throughout the world.[3]

All EHEC strains produce either vero-toxin 1 (VT1) or verotoxin 2 (VT2) or both. The ability to produce verotoxin was acquired from a bacteriophage, presumably directly or indirectly from *Shigella*. These toxins destroy cells by inhibition of the protein biosynthesis. They have affinity for the lining of the colon and the renal glomeruli and tend to initiate life threatening illnesses like Hemorrhagic Colitis (HC) and Hemolytic Uremic Syndrome (HUS), respectively, especially in

those with immune deficiency, young children and the elderly.[3]

Among *Escherichia coli* serotypes that produce verotoxin, O157:H7 serotype is the best known and implicated in food-borne outbreaks worldwide and also responsible for 85-95% of HUS cases. Other EHEC serotypes that are reported occasionally include O111, O26:H11, O103:H2 and O113:H21.[4] The main sources of infection are raw or undercooked meat, alfalfa sprouts, unpasteurized fruit juices, dry-cured salami, lettuce, game meat, cheese curds, raw milk, fresh fruits and vegetables.[5]

As urbanization changed the life-styles, the habit of eating outside at road-sided food stalls is common among the urban population nowadays.[6] The habit of consumption of grilled meat has also been increasing. As VTEC is highly incriminated in undercooked meat, this study was carried out to detect contamination of VTEC in street vended grilled meat.

MATERIALS AND METHODS

It was a cross-sectional, descriptive laboratory-based study conducted from October 2008 to September 2009. A total of 75 grilled meat samples (25 each for grilled chicken, grilled pork and grilled mutton) were collected randomly from street vendors in Latha Township of Yangon Region.

Collection of samples

All the samples were collected aseptically, placed in sterile containers, kept at 4°C and then transferred to the laboratory.

Enumeration of coliforms, fecal coliforms and Escherichia coli

According to standard microbiological analysis procedures,[7] 50 grams of the samples were mixed with 450 ml of Butterfield's Phosphate Buffer and blended for 2 minutes and made three consecutive decimal dilutions and inoculated into triplicate tubes containing Lauryl Tryptose (LT) broth to detect the growth of coliforms. LT-positive tubes were transferred into EC medium and examined for the growth of fecal coliforms. A loopful of suspension from EC-positive tubes were streaked on sorbitol MacConkey agar for isolation of *Escherichia coli* and confirmed by performing biochemical tests (Indole, Voges Proskaure test, methyl-red test and utilization of citrate test).

Toxin detection

VT1 and VT2 were detected by Reverse Passive Latex Agglutination test (VTEC-RPLA kit, Oxoid, TD 960).

Serological characterization

Confirmed *Escherichia coli* isolates were identified for O157 serotype by slide agglutination with O157K+ antiserum (Denka-Seika Company Limited, Japan).

RESULTS

Among 75 grilled meat samples tested, coliforms were isolated from 58 samples (77.33%), fecal coliforms in 45 samples (60%) and *Escherichia coli* in 29 samples (38.7%) (Table 1).

Table 1. Comparison of coliforms, fecal coliforms and *Escherichia coli* contami nation in grilled meat samples

Tested samples	Present (%)	Absent (%)	X^2	P
	Coliforms			
Grilled chicken	17(68)	8(32)		
Grilled pork	23(92)	2(8)	4.72	0.09
Grilled mutton	18(72)	7(28)		
	Fecal coliforms			
Grilled chicken	15(60)	10(40)	0.33	0.846
Grilled pork	16(64)	9(36)		
Grilled mutton	14(56)	11(44)		
	Escherichia coli			
Grilled chicken	11(44)	14(56)	0.79	0.675
Grilled pork	8(32)	17(68)		
Grilled mutton	10(40)	15(60)		

There was no statistically significant relationship between the percentage of contamination of coliform, fecal coliform and *Escherichia coli* among grilled chicken, grilled pork and grilled mutton samples.

Coliform counts of 44 samples (75.9%) were 3-210 MPN/g, 6 samples (10.3%) were 211-500 MPN/g and 8 samples (13.8%) were 501->1, 100 MPN/g. Fecal coliform counts of 38 samples (84.4%) were 3-210 MPN/g, 1 sample (2.2%) was 211-500 MPN/g and 6 samples (13.3%) were 501->1,100 MPN/g. Twenty-four samples showed *Escherichia coli* count of 3-210 MPN/g and 5 samples were 501->1,100 MPN/g (Table 2).

Table 2. Coliform, fecal coliform and *Escherichia coli* counts of grilled meat samples

Tested samples	No. of counts of grilled meat samples			
	<3	3-210	211-500	501->1,100
	Coliform count (MPN/g) (%)			
Grilled chicken	0	9(52.9)	2(11.8)	6(35.3)
Grilled pork	0	20(87.0)	2(8.70)	1(4.3)
Grilled mutton	0	15(83.3)	2(11.1)	1(5.6)
	Fecal coliform count (MPN/g)			
Grilled chicken	0	8(53.3)	1(6.7)	6(40)
Grilled pork	0	16(100)	0	0
Grilled mutton	0	14(100)	0	0
	Escherichia coli count (MPN/g)			
Grilled chicken	0	6(54.5)	0	5(45.5)
Grilled pork	0	8(100)	0	0
Grilled mutton	0	10(100)	0	0

VT1 was detected in 3 isolates (1 isolate from grilled chicken and 2 isolates from grilled pork), VT2 in 3 isolates (2 isolates from grilled chicken and 1 isolate from grilled pork) and VT1+VT2 in 3 isolates (all from grilled pork) (Fig. 1).

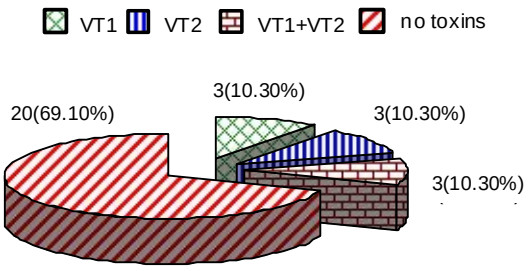

Fig. 1. Percentage of verotoxin producing *Escherichia coli*

Among the isolated *Escherichia coli*, 2 isolates were found to be of O157K+ serotype (1 from grilled pork and 1 from grilled chicken).

DISCUSSION

This study showed that among 75 grilled meat samples tested, *E. coli* was isolated from 29 samples (38.7%) and it is in accordance with the finding of a Thailand study where 38.6% of high heat food samples collected from food vendors was found to be contaminated with *E. coli*.[8] According to New Zealand standard of microbiological reference criteria for food, the recommended microbiological limits of coliforms and *E. coli* for cooked food including meat are 500 and <3 MPN/g, respectively.

In this study, 8 samples (12.1%) showed coliform count of >500 MPN/g and all 29 samples from which *E. coli* was isolated showed *E. coli* count of >3 MPN/g which exceeded the recommended limits. High coliform and *E. coli* counts of the tested samples indicate unsatisfactory hygienic and sanitary standard. Thick sliced meat was found to be more contaminated with bacteria than thin sliced meat probably due to the heat applied being not sufficient to kill the bacteria in the inner part of the meat.

Among 29 *E. coli* isolates, 9 isolates were found to produce verotoxin (VT1 in 3 isolates, VT2 in 3 isolates and VT1+VT2 in 3 isolates) and 2 isolates of VT producing *E. coli* were of O157K+ serotype. O157 serotype has been implicated in many food-borne outbreaks worldwide and is the primary cause of HC and HUS.[4] The infectious dose of EHEC O157 is as low as 2-2,000 cells.[9]

To be fully pathogenic, apart from toxin production, VTEC also require the presence of other virulence markers such as *eae* chromosal gene for attachment and plasmid-encoded enterohemolysin.[3] Although these markers could not be identified, the occurrence of VTEC in this study is of considerable risk for the health of consumers because the morbidity and mortality associated with several outbreaks of VTEC disease have highlighted the threat of these organisms pose to public health.

EHEC O157 strain represents a challenging to prevention of food-borne diseases. Its low infectious dose in combination with disease severity makes successful prevention strategies to be focused on reducing or eliminating the presence of microorganisms, rather than on prevention of pathogen growth. This focus is particularly more important for raw products that may not be thoroughly cooked before consumption (e.g. meat) or ready-to-eat products that do not receive a definitive treatment that assures elimination of *E. coli* O157:H7 (e.g. fermented sausages, apple cider).[3]

Recommendations

- Cook meat thoroughly including the inner part of the meat. Internal temperature should be 160°F.
- Avoid drinking unpasteurized milk and fruit juices. Wash fresh fruits and vegetables thoroughly before eating raw or cooked.
- Separate raw and cooked food and utensils in order to avoid cross-contamination. Keep the food in refrigerator if they are not going to be eaten within 4 hours.
- Health education on the principles of safe food preparation and personal hygiene should be given to general public and food handlers including street vendors.
- Constant monitoring of food and improving diagnostic procedures for detection of VTEC in clinical specimens as well as in foods such as meat and dairy products should be done.
- Cooperation between government, food industries, sellers and the consumers can improve the food safety hygiene.

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REFERENCES

1. Neill MA, Tarr PI, Taylor DV & Trofa AF. *Escherichia coli* cited in Hui, YH, Yorhan JR, Murrell KD & Cliver DO (eds). In: *Handbook on Diseases Caused by Bacteria, Foodborne Disease*. New York. Marcel Dekken. 1994; 169-213.
2. Baljer G & Wieler LH. Enterohemorrhagic *E. coli* (EHEC): Recent information on a new medically important zoonic agent. *Lohmann Information* 1999; (22): 22-26.
3. Buchanan RL & Doyle MP. Food-borne disease significance of *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli*. *Food Technology* 1997; 51(10): 69-75.
4. Griffin PM. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*. In: *Infections of the Gastrointestinal Tract*. Cited in Blaser MJ, Smith PD, Ravdin JI, Greenberg HB & Guerrant RL (eds) New York: Raven. 1995; 739-761.
5. US Food and Drug Administration, Centre for Food Safety and Applied Nutrition. In: *Food-borne Pathogenic Microorganisms and Natural Toxins* 2001, Available from URL: http:// www. fda.gov/ foodsafety/ food-borne illnesses food-borne pathogens natural toxins/bad bug book/ ucm071284.htm, accessed 10 October 2010.
6. WHO commission on Health and Environment. In: *Report of the Panel on Food and Agriculture*, World Health Organization, Geneva, 1992; 87-118.
7. Andrews W. In: *Manual of Food Quality Control 4 Rev. 1*. Microbiological analysis, Food and agriculture Organization of the United Nations, Rome. 1992; 13-24.
8. Chomvarin C, Ratchtrachenchai OA, Chantarasuk Y, *et al.* Characterization of diarrheagenic *Escherichia coli* isolated from food in Khon Kaen, Thailand. *Southeast Asian Journal of Tropical Medicine & Public Health* 2005; 36(4): 931-939.
9. Buchanan RL & Edelson SG. Culturing enterohaemarrhagic *Escherichia* in the presence and absence of glucose as a simple means of evaluating the acid tolerance of stationary-phase cells. *Applied and Environmental Microbiology* 1996; 62: 4009-4013.