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PREVALENCE OF PATHOGENIC ORGANISMS IN MEAT SAMPLES OF ONGKHARAK NAKHONNAYOK THAILAND

M. Vijaya Bhaskara Reddy * and Jitrapun Pusapukdepob

Faculty of Public Health, St. Theresa International College, 1Moo 6, Rangsit, Nakhonnayok Road, Klong 14, Bungsan, Ongkharak, Nakhonnayok - 26120, Thailand.

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Correspondence to Author:
M. Vijaya Bhaskara Reddy

Faculty of Public Health,
St. Theresa International College,
1Moo 6, Rangsit, Nakhonnayok Road,
Klong 14, Bungsan, Ongkharak,
Nakhonnayok - 26120, Thailand.

E-mail: vijaya@stic.ac.th

ABSTRACT: Globally, foodborne pathogens are the origin of diseases and deaths. To prevent the foodborne pathogens billions of dollars spending. Coliforms level, psychrotrophs mesophiles, *E. coli*, and *Staphylococcus aureus*, are routinely assessed to determine microbial safety, sanitation conditions, improper hygiene in poultry carcasses. Thai-population consumes more broiler meat due to its availability and eases to cook, although it could be potential to contaminate with wide varieties of microorganisms. *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, *Campylobacter*, and *Listeria* are the most common food born pathogenic microorganisms. Presence of pathogenic microorganisms in meat could be harmful to human and potential to cause food spoilage. Hence, this can be used as indicator organisms to detect the pathogenicity of food, especially in meat and meat products. Therefore, this study aimed to investigate the prevalence of pathogenic microorganisms, which causes food poisoning in broiler meat as well as discusses concerning public health importance. The findings of this study suggest that the consumption of cross-contaminated meat with pathogenic organisms may pose a serious threat to local consumers. *E. coli* and *Salmonella* was observed at higher prevalences in chicken meat samples collected from raw meat sellers of the study area. The results of the study provided concrete evidence on zoonotic transmission of the pathogenic organisms to human.

INTRODUCTION: Majority of Thai-population consumes broiler meat due to its availability and ease to cook, although it could be potential to contaminate with wide varieties of microorganisms. *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, *Campylobacter*, and *Listeria* are the most common food born pathogenic microorganisms [1]. In general, pathogens tend to be disseminated during the different stages of slaughtering processing [2].

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According to global epidemiological studies indicates that broiler meat and poultry products play an essential role in food poisoning [3]. Prevalence of spoilage and pathogenic organisms in poultry meat and its products remain a remarkable concern for suppliers, retailers, consumers [4, 5] as well as poultry producers and global public health professionals.

Poultry meat and its products are often infected with different pathogenic organisms such as *E. coli*, *Salmonella* and can be transmitted to mammals either handling of carcasses or consumption of uncooked meat [6]. *Salmonella* typically occurs in poultry, and its products, especially meat products, are concerned as a vector for foodborne diseases. Worldwide, salmonellosis is one of the most frequently reported foodborne diseases [7].

Campylobacteriosis, mainly occurs due to animal origins especially meat and its products play a key role in the dissemination of campylobacteriosis in humans. In the United States of America, *C. jejuni* reported as common causative of foodborne infections and has been emerged as the most common cause for bacterial gastroenteritis in humans [8]. Coliforms level, psychrotrophs mesophiles, *E. coli*, and *Staphylococcus aureus*, are routinely assessed to determine microbial safety, sanitation conditions, improper hygiene in poultry carcasses.

Globally, foodborne pathogens are the origin of diseases and deaths. To prevent the foodborne pathogens billions of dollars spending [9]. *E. coli* is one of the common origins of foodborne diseases in mammals. Resistant strains of *E. coli* infect at all the age groups, and acid resistance, a wide range of infections are the severe consequences [10]. The severity of the infections depends on the host susceptibility, virulence of the *E. coli*, and dose. Prevalence of resistant strains of *E. coli* results in mild and severe bloody diarrhea, hemorrhagic colitis, and or hemolytic uremic syndrome, which lead to kidney failure [10, 11]. Livestock animals are the primary reservoirs of *E. coli*, and meat products are determined ass major root cause of foodborne transmission [10, 12]. Carcass contamination occurs through skin-to-carcass or fecal-to-carcass transfer of the pathogen during the slaughter process at processing plants [13, 14, 15]. These are the major concerns for human infection. However, during the processing of meat direct, indirect and or cross-contamination may occur. Antimicrobials are using mainly reduce pathogen shedding [16, 17] and washing of skin and carcass [18].

For the first time, by using genetic fingerprinting techniques, Kudva *et al*.,[19] found multiple strains of *E. coli* O157: H7 in a single flock of sheep and showed that a single animal shed multiple strains simultaneously and that strains shed by individuals changed over time. *Escherichia coli* O157: H7 has been isolated from animal drinking water, animal feed, flies, and a pigeon at dairy farms in Wisconsin [20]. The majority of isolates collected at these farms had the same genetic fingerprint. Although various methods are available for genetic characterization of bacterial isolates, random amplification of polymorphic DNA (RAPD) has been used successfully in the past for *E. coli* O157: H7 [21, 22, 23] and is less costly and time-consuming than other methods. As a source of animal protein, goat meat has for long occupied a special place in the diet for a variety of reasons including taste preference, prestige, religion, tradition, and availability, in almost all the communities of the country with the nutritional aspects being included more recently. The meat was the first important food that met up the hunger of ancient people living in cave [24]. It plays a very vital role in keeping the human body strong to provide energy and health [25]. But, the presence of pathogenic microorganisms in meat could be harmful to human and potential to cause food spoilage.

Hence, this can be used as indicator organisms to detect the pathogenicity of food, especially in meat and meat products. Many researchers have isolated and identified heterogeneous types of microflora from fresh meat, recently reported in Ongkharak, Nakhonnayok, Thailand [26]. Plasmid analysis has also proved a useful method for differentiating bacterial isolates [27, 28]. The number and size of the plasmids present are used as the basis for strain identification. This strain typing technique has been used successfully for the analysis of outbreaks of nosocomial infections [29] and community-acquired infections [30] caused by a variety of species of gram-negative rods. Therefore, this study aimed to investigate the prevalence of pathogenic micro-organisms, which causes food poisoning in broiler meat as well as discusses concerning public health importance.

MATERIALS AND METHODS:

Samples Collection: A total of 1105 samples were collected from chicken broiler from processing plants and retail shops in Ongkharak Nakhonnayok, Thailand. Samples were wrapped in a sterile polyethylene bag and identified. The collected carcase samples were transported immediately to the laboratory under controlled conditions.

Sample Preparation: Skin and muscle samples of neck, breast and thigh skin and muscle samples include breast and thigh muscle. 10 g samples were collected aseptically from each category followed by ICMSF [31]. The bacterial count was carried out according to APHA [32]. Coliforms most probable number (MPN) was conducted by three tubes

protocol, fecal coliforms most probable number (MPN), *E. coli* most probable number (MPN) and *S. aureus* count.

Bacterial Isolation: *Salmonella* isolation according to ISO 6579:2002 [33], *E. coli* isolation according to APHA, *Staphylococcus aureus* isolation according to APHA [32], & *Campylobacter* isolation according to ISO 10272-1: 2006 [34].

RESULTS: Out of 1105 samples 416(75.6%) of *E. coli* isolated from chicken meat processing plant and retail shops and 51.11%, 30.37%, 48.88%, 48.14% of *E. coli* isolated from faecal, skin swabs, intestinal mucosa, and environmental samples collected from processing plant with an overall prevalence of 44.63%. Whereas samples from retail shops such as carcass (50.83%), hands (24.16%), knife (30%), cutting board (37.50%), and health centers (4.70%) with 30.97% of overall prevalence of *E. coli* in samples collected from retail shops **Table 1**. The highest prevalence of *E. coli* strains in the study area suggests that the high concern for the community health.

Environmental sampling attributed as a useful method to check whether it has any impact on the persistence and dissemination of *E. coli* strains. Prevalence of *E. coli* in meat and environmental samples reveals the detrimental effects of *E. coli* and the importance of hygiene of food products.

TABLE 1: PREVALENCE OF *E. COLI* FROM DIFFERENT SAMPLES OF PROCESSING PLANTS, RETAIL MARKETS AND HEALTH CENTERS IN ONGKHARAK, THAILAND

Source of the sample	Type of sample	Samples examined (n)	Positives (n)	Frequency (%)
Processing plants	Fecal	135	69	51.11
	Skin swab	135	41	30.37
	Intestinal mucosal swab	135	66	48.88
	Environmental swab	135	65	48.14
The overall prevalence in processing plants		540	241	44.63
Retail shops	Carcass	120	61	50.83
	Hands	120	29	24.16
	Knife	120	36	30.0
	Cutting board	120	45	37.50
	Health centers	85	4	4.70
The overall prevalence in retail shops		565	175	30.97

TABLE 2: PREVALENCE OF *SALMONELLA* FROM DIFFERENT SAMPLES OF PROCESSING PLANTS, RETAIL MARKETS AND HEALTH CENTERS IN ONGKHARAK, THAILAND

Source of the sample	Type of sample	Samples examined (n)	Positives (n)	Frequency (%)
Processing plants	Fecal	135	56	41.48
	Skin swab	135	42	31.11
	Intestinal mucosal swab	135	29	21.48
	Environmental swab	135	89	65.92
The overall prevalence in processing plants		540	216	40.0
Retail shops	Carcass	120	49	40.83
	Hands	120	65	54.16
	Knife	120	39	32.50
	Cutting board	120	44	36.66
	Health centers	85	6	7.06
The overall prevalence in retail shops		565	203	35.92

Out of 1105 samples 419(75.92%) of *Salmonella* isolated from chicken meat processing plant, retail shops, and 41.48%, 31.11%, 21.48%, 65.92% of Salmonella isolated from fecal, skin swabs, intestinal mucosa, and environmental samples collected from processing plant with an overall prevalence of 40%. Whereas samples from retail shops such as carcass (40.83%), hands (54.16%), knife (32.50%), cutting board (36.66%), and health centers (7.06%) with 35.92% of overall prevalence in samples collected from retail shops **Table 2**.

The higher levels of *Salmonella* in the study area suggests that the high concern for community health. Prevalence of *Salmonella* in meat and environmental samples reveals the detrimental effects of food poisoning microorganisms and the importance of hygiene of food products.

TABLE 3: PREVALENCE OF *STAPHYLOCOCCUS AUREUS* FROM DIFFERENT SAMPLES OF PROCESSING PLANTS, RETAIL MARKETS AND HEALTH CENTERS IN ONGKHARAK, THAILAND

Source of the sample	Type of sample	Samples examined (n)	Positives (n)	Frequency (%)
Processing plants	Fecal	135	39	28.88
	Skin swab	135	54	40.0
	Intestinal mucosal swab	135	47	34.81
	Environmental swab	135	61	45.18
The overall prevalence in the processing plant		540	201	37.22
Retail shops	Carcass	120	56	46.66
	Hands	120	8	6.66
	Knife	120	28	23.33
	Cutting board	120	36	30.0
	Health centers	85	4	4.70
The overall prevalence in retail shops		565	132	23.36

Out of 1105 samples 333(60.58%) of *S. aureus* from chicken meat processing plant, retail shops, and 28.88%, 40%, 34.81%, 45.18% of *Staphylococcus aureus* isolated from fecal, skin swabs, intestinal mucosa, and environmental samples collected from processing plant with an overall prevalence of 37.77%. Whereas samples from retail shops such as carcass (46.66%), hands (6.66%), knife (23.33%), cutting board (30%), and health centers (4.70%) with 23.36% of overall prevalence in samples collected from retail shops **Table 3**. The higher levels of *Staphylococcus aureus* in the study area suggest that the high concerns for the community health and need to take initiatives to prevent the food poisoning pathogens. Prevalence of *Staphylococcus aureus* in meat and environmental samples shows the importance of hygiene of food products.

TABLE 4: PREVALENCE OF *CAMPYLOBACTER JEJUNI* FROM DIFFERENT SAMPLES OF PROCESSING PLANTS, RETAIL MARKETS AND HEALTH CENTERS IN ONGKHARAK, THAILAND

Source of the sample	Type of sample	Samples examined (n)	Positives (n)	Frequency (%)
Processing plants	Fecal	135	47	34.81
	Skin swab	135	38	28.14
	Intestinal mucosal swab	135	61	45.18
	Environmental swab	135	29	21.48
The overall prevalence in processing plants		540	175	32.40
Retail shops	Carcass	120	27	22.50
	Hands	120	54	45.0
	Knife	120	4	3.33
	Cutting board	120	16	13.33
	Health centers	85	2	2.35
The overall prevalence in retail shops		565	103	18.23

Out of 1105 samples 278(39.718%) of *C. jejuni* from chicken meat processing plant, retail shops, and 34.81%, 28.14%, 45.18%, 21.48% of *Campylobacter jejuni* isolated from faecal, skin swabs, intestinal mucosa, and environmental samples collected from processing plant with an overall prevalence of 32.40%. Whereas samples from retail shops such as carcass (32.406%), hands (45.0%), knife (3.33%), cutting board (13.33%), and health centers (2.35%) with 18.23% of overall prevalence in samples collected from retail shops **Table 4**. The higher levels of *Campylobacter jejuni* in the study area suggests that the high concerns for the community health and need to take initiatives to prevent the food poisoning pathogens and the importance of hygiene of food products.

DISCUSSION: The results of the study provided concrete evidence in food pathogenic organisms **Table 1**, **2**, **3** and **4** similar result was reported [35], whereas higher prevalence was reported by Bhandari *et al*., [36]. On the other hand, lower levels of coliform counts were reported by Buhr *et al*., [37] in breast skin, neck skin [38], breast [39] and thigh muscles of chickens [40]. Elevated levels of coliforms may be attributed as live birds and animals are hosts to a wide variety of microorganisms residing on their skin, feathers or in the alimentary tract. In generally, the slaughtering process of birds is considered as highly contaminated with bacteria. Most of these pathogenic microorganisms are eliminated during slaughter.

Subsequently, contamination may occur at any of these stages such as production process, feather plucking, evisceration and washing, environment, equipment, freezing, and workers hands can cause contamination of meat and its products. Similar findings have been reported by Kotula and Pandya [41] and Geornaras *et al*., [42].

Prevalence of coliform bacteria, fecal coliforms is good microbial indicators of the potential presence of disease. Fecal coliforms had been used as an indicator for fecal contamination. Fecal contamination may occur due to the contaminated carcasses from the gut of slaughtered birds and later which passed on as contaminants. Improper evisceration may lead to a significant increase in carcass contamination. The results of this study by the reports of Russell and Walker [43], Adeyanju and Ishola [44], similarly *E. coli* [45] and Berrang *et al*., [46], Cohenet [47] in chicken meat, in skin [37, 38, 40, 41, 42]. The isolated serotypes were O157 and O18 from chicken.

Adesiji *et al*., [47] found and reported that *E. coli* has been isolated worldwide from poultry meat. Elevated level isolates of *E. coli* were reported 48 and he reported that 90% isolated *E. coli* from breast and 100% from thigh skin respectively, in another study Saikia and Joshi [49] reported that 98% *E. coli* was isolated from chicken meat and Odwar *et al*., [50] who reported that 78% contamination by *E. coli* in chicken meat. On the contrary lower levels of *E. coli,* isolates were reported [44, 47]. *E. coli* is a natural inhabitant of the intestinal tracts of warm-blooded animals and humans. This could be used as an indicator of the presence of the bacterium.

Prevalence of these micro-organisms reflects contamination and indicates possible contamination of the enteric pathogen. Raw or uncooked food stuff get contaminated either production, slaughtering or handling, processing, cross contamination (human-to-food contamination) [44]. After, slaughtering poultry carcasses scalded in a common scaling tank in retail poultry meat shops, under poor hygienic conditions such as stagnant water, excreta and or non-bactericidal temperatures, *etc*., these conditions serve as a transmitter for contamination, and finally, contamination disseminates to all the birds [51].

Staphylococcus aureus was isolated from various samples of chicken meat processing plant, retail shops, and 28.88%, 40%, 34.81%, 45.18% of *Staphylococcus aureus* isolated from fecal, skin swabs, intestinal mucosa, and environmental samples collected from processing plant with an overall prevalence of 37.77%. Whereas, samples from retail shops such as carcass (46.66%), hands (6.66%), knife (23.33%), cutting board (30%), and health centers (4.70%) with 23.36% of overall prevalence in samples collected from retail shops **Table 3**. Similar results were reported [52, 53, 54, 55]. Elevated levels were reported [56, 57, 58, 59].

The higher prevalence rates of *E. coli* and *Staphylococcus aureus* was observed in this study. The higher prevalence rates of *Staphylococcus aureus* and *E. coli* could be attributed to the poor hygienic conditions and improper usage of techniques to open the abdomen of the birds. However, hand evisceration predominantly affects the hygiene of the workers as well as the surrounding environment. The high prevalence rate of specific pathogenic microorganisms interlinked with poor hygiene and human contact. The occurrence of *Salmonella* indicates the poor hygienic practices during slaughtering and processing of meat [60, 61, 62].

The results of this study revealed and provided concrete evidence on how poor hygienic practices could influence the quality and hygiene of poultry meat. The pathogenic organisms can be disseminated through the work environment, intestinal contents, skin, carcasses, and knives. The results of this study by the previous studies [63, 64, 65, 66]. On the contrary, some reported lower levels [67, 68]. Poultry meat often infected through poor hygiene of carcasses and undercooked poultry products [69, 70]. Presence of pathogenic micro-organisms in meat could be harmful to human and potential to cause food spoilage. Hence, this can be used as indicator organisms to detect the pathogenicity of food especially in meat and meat products [26].

Salmonella, a most inculpated food poisoning pathogen which often found in poultry meat attributed as increasing the public health concerns. Because it can act as a symbiotic organism as well as adisease-producing pathogen. *Salmonella* can

cause a wide range of diseases such as skin, bone and joint infections, food poisoning, bacteraemia, medical implant implications, and act as virulent factor. The salmonellosis is emerging and remerging incidence in human health, due to these reasons poultry infections alarms the public health professionals. The poultry industry and its products are the major reservoirs for salmonella. The prevalence of *Salmonella* in chicken meat may be attributed as an outcome of intestinal cross-contamination, poor hygienic procedures, unorganized retail marketing, and water which used for washing of hands, carcasses, containers as a whole all these could be contaminated through feces or cross contamination. The study revealed that raw chicken meat was often contaminated with salmonella and *E. coli* in retail markets of Ongkharak, Nakhon Nayok Thailand. Poultry meat often infected with pathogenic microorganisms through poor hygiene of carcasses, production premises, surrounding environment and undercooked poultry products.

CONCLUSION: The findings of this study suggest that the consumption of cross-contaminated meat with pathogenic organisms may pose a serious threat to local consumers. *E. coli* and *Salmonella* was observed at higher prevalences in chicken meat samples collected from raw meat sellers of the study area. It indicates the transmission of the pathogenic organisms by the animal to human. Results suggest that the high prevalence of *E. coli* and *Salmonella* in chicken meat may be due to current sanitary systems at processing units and retail shops. Further, epidemiological studies need to be undertaken on production and processing systems in poultry to substantiate the findings of the study.

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