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SALMONELLA CONTAMINATION IN DIETARY MEAT SOURCE IN MALAYSIA

Pravin Raj Solomon* **Sarbeshwaary Ramakrishnan****
**Faculty of Biomedical Sciences, MAHSA University, Malaysia.*
***School of Life Sciences,Northumbria University.*

Abstract
Salmonella contamination is a leading cause of certain food borne diseases and therefore targeted foods are to be screened. Skin, liver and muscle parts of halal and non-halal chicken, beef, lamb purchased from Kepong and Sentul wet market, KL, Malaysia during the month of October 2014 were tested for salmonella contamination. Salmonell account in the non-halal meat sample is higher compared to that of halal meat. Salmonella count shows relatively higher infestation in chicken, followed by beef and lamb. Skin and liver samples from halal and non-halal meat are free from Salmonella but muscle samples suffer contamination. Lemon, and NaCl solution application reduced Salmonella contamination to a greater extent. Prevention of Salmonella contamination in dietary meat source is a basic step in food safety control. Improper cleaning, storage and insufficient cooking cause Salmonellasp.to survive and cause cross-contamination. Hence, preserving meat with proper preservative becomes an essential step in controlling the contamination.

Keywords: Meat, Salmonella, Lemon Extract, Sodium Chloride.

Introduction

Salmonellais a major component of the gut micro flora of animals, including humans and other animals such as birds, reptiles, amphibians which are richly found in their feces. Fecal pollution is the main route by which food and water supplies become contaminated and largely account for the ubiquity of Salmonella in the food supply chain. Poultry and pigs, can also become infected and act as reservoirs of Salmonella.

One of the more important microbes in the family *Enterobacteriaceae*is the genus *Salmonella, a gram negative bacteria.*The genus *Salmonella* was previously differentiated in to two species, *S.enterica*and *S.bongori***(**Ozkalp, 2012**).** However, a new species, *S. subterranea*was identified and validated. According to White-Kauffmann-Le Minor Scheme *S. enterica*comprises six sub species, *S. enterica*sub sp. *enterica*, *S. enterica*sub sp. *salamae*, *S. enterica*sub sp. *arizonae*, *S. enterica*sub sp. d*iarizonae*, *S. enterica*sub sp. *houtenae*, and *S. enterica*sub sp. *indica*. Formerly, *S. bongori*was the sub species , but later considered as a separate species **(**Foley *et al*.,2013**).** Currentlythere are over 2,600 *Salmonella* serotypes, in which 99 percent of these serovars are in *S. enteric* and almost 60 percent belong to *S. enteric* sub sp. *enterica***(**Pui*et al*., 2011**)**. However, out of these six sub species, only subspecies *S. enterica* is associated with disease in warm-blooded animals **(**Porwollik*et al.*, 2004**).**

Most of the *Salmonella* isolates concerned with human infection belong to *S. enterica*sub species *enterica***.**In European Union (EU), *S. enteritidis*and *S. typhimurium*serotypesare reported to be the major etiological agents of salmonellosis which have adapted to humans **(**Hendriksen*et al.,* 2009**).** *S.enterica*serover*enteritidis*and *S. enterica*serover*typhimurium*are the frequently encountered species in the tissues of poultry, pork and beef product.

Salmonellosis leads to enteric (typhoid) fever, colitis and systemic infections. Individuals infected with enteric fever either from the *S. typhi*or *S. paratyphi*strain exhibit severe and serious symptoms (Zhang *et al.,* 2003).Impacts of such clinical conditions are high in newborns, infants, the elderly and immune compromised individuals. Ingestion of even 10 colony forming units (Cfu) of *S. typhimurium*from common foods, such as cheddar cheese or chocolate may cause illness. Salmonellos iscaused by the penetration and passage of *Salmonella* cells from the gut lumen in to the epithelium of the small intestines, predominately from a contaminated food source (Kaur and Jain, 2012**).**

Outbreaks of food borne salmonellosis are common and are widely disseminated through food (Anonymous, 2011). In Malaysia, as high as 57% of diarrheal cases among children due to Salmonella was reported (Kit *et al.,* 2011). The National Antibiotic Resistance Surveillance programme revealed that in 2000, more than 8.5% of *S.typhi*was detected from meat in Malaysia (Lim, 2002).The incidence of *Salmonella* contamination in poultry meat forms a major source of pathogen and the rate of infestation gradually increases every year (Kaushik*et al.,* 2014).

In Malaysia, the meat products are grouped as halal (Kosher) and non-halal product (Non-Kosher). All blood must be removed from the tissues of a meat or poultry product to be considered kosher. In addition, halal has become popular among Muslim consumers in the world (Zailani*et al.,* 2010).Halal meat is from the animals slaughtered according to Islamic law, in

which the animal is killed by a transverse cut to the throat and the blood is allowed to drain. Genuine halal meat is sold from registered shops and is often eaten within 24 hours of slaughter. In such process, the chances of bacterial contamination is less as during the slaughter, the blood loaded with microbes is drained together with the blood and less pathogens will be present in the meat since many studies indicate that blood is the principal habitat for the microbes to multiple. Therefore, this work aims to compare the *Salmonella* count in the halal and non-halal meat products.

Organic acids are often used as antimicrobial acidulates to preserve foods either by direct addition or through microbiological fermentation. Since the multiplication of pathogens is arrested at pH below 4.5, acidification may act to prevent microbial proliferation. Low pH disrupts the membrane transport or permeability causing anion accumulation or a reduction in internal cellular pH due to the dissociation of hydrogen ions from the acid (Ricke*et al.,* 2013).

Therefore the present work is designed to identify Salmonella contamination in various meat products being marketed. This work aims at to compare theSalmonella distribution between halal (Kosher) and non-halal meat and to evaluate the effect of storage condition in Salmonella contamination. Attempts are made to determine the effect of the natural preservatives such as lemon juice and sodium chloride on the population of *Salmonella*sp .in meat product.

Materials and Methods

Sample collection

Halal and non-halal meat samples (chicken, beef, lamb) containing skin, liver and muscle parts were purchased freshly .The samples were collected aseptically in sterile polythene bags**.** They were transported to the laboratory and processed within 1 h of collection to minimize the microbial changes due to environmental temperatures and post-slaughter timings.

Quantification methods to determine Cfu g^{-1}

Each meat sample(25g) was weighed and blended. A gram of the above blend was weighed aseptically and homogenised in 9 mL of sterile buffered peptone water as pre-enrichment medium and a six-fold serial dilutions were prepared (10^6) .From that 0.1ml of appropriate dilutions were cultured by spread plate technique using sterile bent glass rod on the Salmonella-Shigellaagar and then incubated overnight at 37C° for 24 h. Colony count in the plate between 30 and 300 are considered to be statistically acceptable (Adeyanju and Ishola, 2014).

Effect of Natural Preservatives

1g homogenate of each muscle sample was mixed with 1mL of lemon juice and 1 mL of 1, 1.5, 2 and 2.5% of sodium chloride separately and stored for different time duration(30 min to 1 h). One ml of the above was transferred in to 9 mL of sterile buffered peptone water as pre-enrichment medium and a six-fold serial dilutions were prepared (10^6). 0.1ml of each appropriate dilution were cultured by spread plate technique using sterile bent glass rod on the Salmonella-Shigellaagar and then incubated overnight at 37C° for 24 hours. Then, the *Salmonella*sp.suspected colonies were subjected to biochemical test (IMViC) and API 20E for confirmation.

One loop full from each of the enriched broths were streaked on to plates of Salmonella Shigellaagar (SS), Xylose Lysine Deoxycholate (XLD) agar , Brilliant green (BGA) agar, Bismuth Sulfite (BSA) agar and incubated at 37 °C for 18 -24 hours (WHO, 2010). After incubation, the plates were examined for the presence of typical colonies of *Salmonella* sp.

The two microbial ATCC stains consisting of Positive control and Negative control were used in this study. The ATCC bacteria were first ensured for purity by culturing the bacteria onto MacConkey agar and Nutrient agar**.** The plates incubated at 37 °C for 18 -24 hours. After incubation, typical colonies were examined for their colony characteristics, gram stain morphology and biochemical test . The typical colonies were smeared and stained with gram's stain and examined morphologically for staining characteristics (WHO, 2003). The presumptive colonies were biochemically tested by IMViC test such as indole (I), Methyl Red (M), VogusProskeur (Vi),Citrate (C), Triple sugar test (TSI) and Urease Test. All the cultures were incubated at 37°C for 18 -24 hours and validated by the positive control (*Salmonella*sp.) and negative control (*E.coli*).

Suspected colonies of *Salmonella spp.* were identified by Gram staining performed according to the conventional method and IMViC test with API 20E test kit to further confirm the microorganism present. The plastic strips holding twenty mini-test tubes were inoculated with the saline suspensions of the cultures as per the manufacturer's directions. This process rehydrated the desiccated medium in each tube. A few tubes were completely filled (CIT, VP and GEL), and some tubes were overlaid with mineral oil such that anaerobic reactions could be carried out (ADH, LDC, ODC, H2S, URE). After incubation in a humidity chamber for 18-24 h at 37°C, the colour developed were read (some with the aid of added reagents

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as supplied by the kit). The data were analysed by the manufacturer's software and positive results with ≥ 89% probabilities were confirmed as *Salmonella* sp.(Ozkalp, 2012).

Result

The occurrence of Salmonella in various muscle parts of chicken, beef and lamb is given in fig.1.The population density of the Salmonella in the muscle of halal meat is chicken > beef > lamb. Similarly in non–halal meat the occurrence is chicken > lamb.

In 1percent lemon (*ie* 1 mL of lemon juice) the colonies was completely suppressed. Similarly when the concentration of sodium chloride increases, more number of *Salmonella*sp.colonies were inhibited. Hence in lemon juice and in 2-2.5percent sodium chloride the salmonella is completely inhibited and the meat sample is free of *Salmonella*sp.contamination.

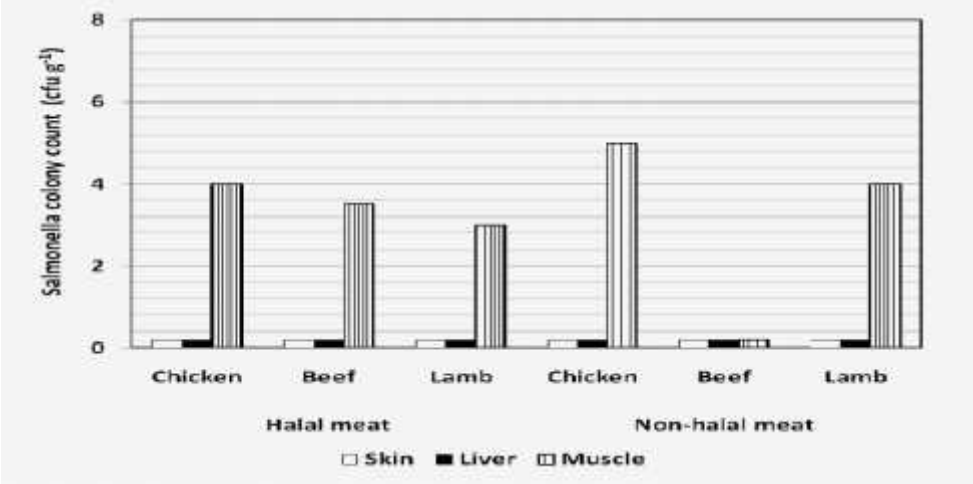

Fig.1:Salmonellacolony count(cfu g^{-1}) in halal and non-halal meat samples.

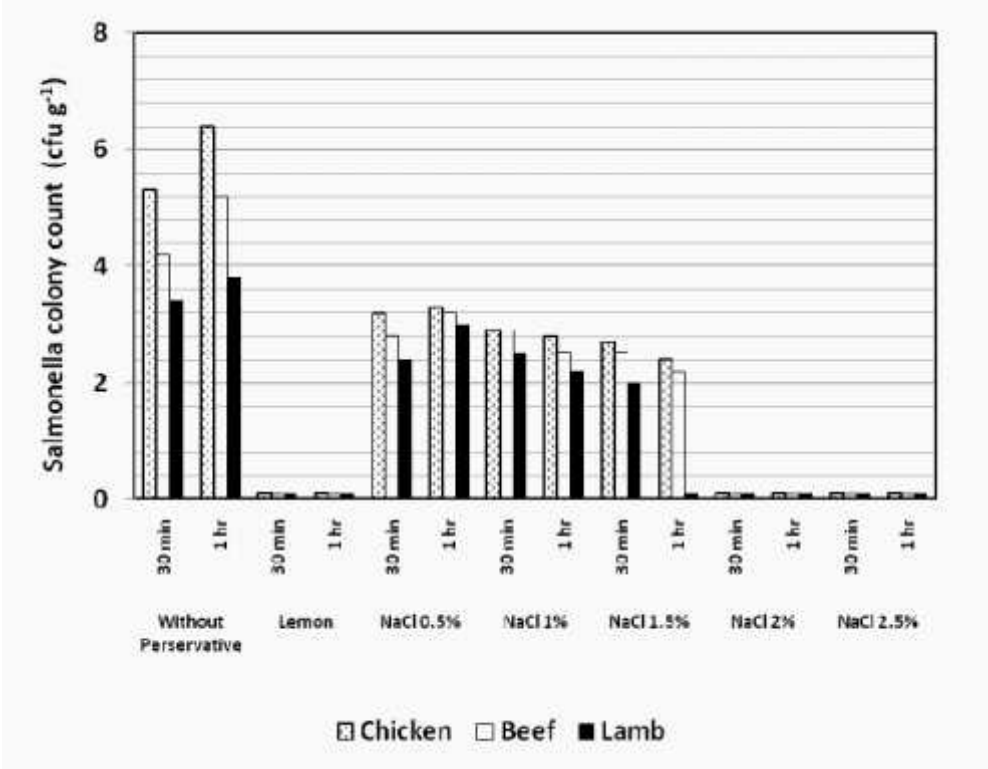

Fig.2:Salmonellacolonies count(cfug^{-1})of meat samples in various preservatives

Discussion

Salmonellosis is considered as one of the anthropozoonotic diseases of serious medical implications which raises serious concern in food industry. Meat is considered as a source of *Salmonella* infections in human and is a potential source of food poisoning in man.

Salmonellosis reported worldwide became an important public health problem. Nearly, 1.4 million cases of salmonellosis occur each year in the United States. The incidence of *Salmonella* infections has risen dramatically in Europe since the 1980s (Brands *et al.*, 2005).

In this study, it known that the *Salmonella* count in the non-halal meat is higher when compared to that of halal meat. In halal meat, the animal is slaughtered according to Islamic law, in which the animal is killed by a transverse cut to the throat and letting the blood completely drained off. For non halal meat, the animal is slaughtered by many ways and there is possibility that the blood is not completely drained off. In the halal meat, the chances of bacterial contamination are less.

The result also indicates that among all the three types of meat (chicken, beef and lamb), the *Salmonella* show higher presence in the chicken, followed by beef and lamb. *Salmonella* often found on chicken sample since chickens peck at droppings and ingest them. The bacteria settle in their intestines, usually without causing any harm, and the chickens contaminate their environment with infected feces. When the birds are slaughtered, intestinal bacteria can wind up on their carcasses. Therefore, inadequate cleaning of the meat and improper storage and insufficient cooking cause *Salmonella* to survive and people may encounter these microbes through cross-contamination from an infected individual. The chicken meat is one of the major food sources of protein in the food industry and therefore controlling *Salmonella* contamination is essential in chicken meat (Hendriksen*et al*., 2009). The muscle sample is more prone to *Salmonella* contamination compared to other tissue as muscle is a most potential source of contamination with *Salmonella*. This happens because after the animal is slaughtered the glycogen in the muscle is converted into lactic acid causing a fall in pH from an initial value of pH 6.8 - 7.3 to about 5.4 - 5.8. Such meat having a pH around 6.0 spoil quickly since the low acidity favours rapid bacterial growth.

The skin and liver of both halal and non-halal sample is free of *Salmonella* contamination because skin layer interfaces with the environment and is the first line of defense from external factors. In some animals such as chicken and lamb, skin is very hard and thick and has hard protective scales on their skin for protection. Hence, it is able to protect the pathogens from entering the body through skin-contact mode. While, the result shows, the skin are free of *Salmonella*sp.contamination because *Salmonella*sp.is fastidious organism and mostly survive in the blood and contaminate the meat via cross contamination (Zhang et al., 2003).

In addition, to regular cleaning preservatives used on the meat sample avoid any spoilage or rotting and consequently the texture of the meat is maintained. The preservatives inhibit or damage the Salmonellacells present in the surface such as skin. Liver being the detoxifying apparatus the chances of *Salmonella* occurrence in the liver is less when compared to that of other parts. Preservative is considered essential to store or preserve the meat .When the meat is kept at room temperature without any preservative, the *Salmonella* count increases. Therefore, pre-processing the meat in a simple preservative is considered appropriate especially when the cooking is delayed.

Lemon juice is considered as antimicrobial agent to preserve foods either by direct addition or through microbiological fermentation. As the growth of pathogens are slowed down at pH values below 4.5 due to lemon juice acidification. Lemon juice contains citric acid which has the highest inhibitory effect because of its ability to diffuse through the cell membrane. Hence, by preserving the meat with lemon juice showed significant antibacterial activity against food borne pathogens commonly implicated in meat processing. Lemon also is a powerful antioxidant which is known to prevent spoilage and rotting .Hence lemon juice is considered to enhance the shelf-life of packaged meat significantly. The results suggest that marinating the chicken in lemon juice can greatly reduce populations of pathogenic bacteria, thus enhancing overall food safety and shelf life of chicken meat. Sodium chloride also is used commonly in food products to improve the shelf life. Adding common salt to foods may bring in osmotic shock resulting in the loss of water from the cell and thereby causing cell death or retarded growth. The result shows total *Salmonella* colonies count ($cfug^{-1}$) is decreased as the concentration increases. *Salmonella* has not been able to grow in high concentration(2-2.5%) of sodium chloride because high concentration of salt limits oxygen solubility, interfere with cellular enzymes, or force cells to expend energy to exclude sodium ions from the cell, all of which can ultimately arrests the proliferation of the microbes. (Fig.2)

Food-borne salmonellosis often follows consumption of contaminated meat from infected animals or from contamination of the carcasses or organs during slaughtering. Cutting board used in the preparation of meat and grinders, miners, and blenders are considered as an important source of meat contamination by *Salmonella*. Besides tracks,lairages, slaughter line,

quartering, knives and surface of table are main sources of *Salmonella* contamination in meat and meat products. Microbiologically polluted water when used to clean equipment and machines leads to cross-contamination of the meat. Therefore *Salmonella* infection needs to be targeted since it indicates that it is one of the most common food borne infection. This study is able to generate the data on *Salmonella* contamination in meat and the possible way of controlling the exigencies very effectively.

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