IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 8, Issue 3 Ver. I (Mar. 2015), PP 24-27
www.iosrjournals.org

Assessment of Microbial load of raw meat Samples sold in the Open Markets of city of Kolkata

Keshab Prasad Sharma[1*] and U. K. Chattopadhyay[2]

[1] *M.V.P.H Scholar, Department of Microbiology, All India Institute of Hygiene & Public Health, JC-27, Sector-III, Kolkata-700098, India.*

[2] *Director- Professor & Head, Department of Microbiology, All India Institute of Hygiene & Public Health, JC-27, Sector-III, Kolkata-700098, India.*

Abstract: *The present investigation was undertaken to assess the microbial load of raw meat samples collected from open markets of the city of Kolkata. In the present investigation 200 raw meat samples (100 chickens and 100 muttons) were utilized.* In this study, the pathogenic microorganisms were isolated in different percentages viz. *E. coli* (98%), *Enterococcus faecalis* (90%), *Staphylocoocus aureus* (20%), *Staphylococcus epidermidis* (20%), *Pseudomonas spp.* (10%), *Salmonella spp.* (2%), *Bordetella* (1%). Other organisms that were isolated in this study were *Klebsiella pneumoniae* (98%), *Enterococcus aerogenes (*90%), *Micrococcus spp.* (69%), *Citrobactor spp. (52%), Proteus spp. (50%), Klebsiella oxytoca (35%) and Providencia spp. (22%).* This has a definite implication from the Public Health point of view. The findings indicate substantial presence of microbial contaminants in chicken and mutton meat in open markets of Kolkata where dearth of proper sanitation in the market places prevails.

Keywords: *Assessment, Kolkata, Meat, Microbial load, Open market.*

****Corresponding Author***: Keshab Prasad Sharma. E-mail: ***Kepash08@gmail.com***
Dispur Last Gate, Capital Complex, Guwahati-781006, Assam.

I. Introduction

World population is growing very fast. The increase in population with consequent pressing demand for enhanced requirements of food has led to a continued search for novel sources of food and protein. It is, therefore, of immense importance to look for alternative sources of foods for catering to the demands in the years to come as there are no additional land masses to be discovered that would be available for traditional agriculture. Ruminants are probably the most efficient converters of plants into muscles because their digestive system can utilize cellulose and fibrous materials, the most abundant raw food material on the earth, and even non-protein nitrogen. Ruminants convert the materials into a well balanced source of protein and energy for human consumption called Meat. Meat, an excellent source of protein in human diet is highly susceptible to microbial contaminations, which can cause its spoilage and food borne infections in human, resulting in economic and health losses [1]. Although muscles of healthy animals do not contain microorganisms, meat tissues get contamination during the various stages of slaughter and transportation [2]. A great diversity of microbes inhabit fresh meat generally, but different types may become dominant depending on pH, composition, textures, storage temperature, and transportation means of raw meat [2,3,4]. Raw meat may harbour many important pathogenic microbes i.e. *Salmonella spp*., *Campylobacter jejuni/coli*, *Yersinia enterocolitica*, *E. coli*, *S. aureus* and, to some extent, *Listeria monocytogenes*, making the meat a risk for human health, as without the proper handling and control of these pathogens, food borne ill-nesses may occur [5]. Meat can be contaminated at several points throughout the processing operations. Moreover, retail cuts could result in greater microbial load owing to large amount of exposed surface area, more readily available water, nutrient and greater oxygen penetration which leads to spoilage of meat. Meat borne zoonotic diseases such as Salmonellosis, Campylobacteriosis, E. coli enteritis and food poisoning by Clostridium, Staphylococcus, etc. are the major problems encountered by the consumers eating contaminated meat. Numerous epidemiological reports have implicated foods of animal origin as the major vehicles associated with illnesses caused by food-borne pathogens [6, 7]. Contaminated raw or undercooked poultry and red meats are particularly important in transmitting these food-borne pathogens [8]. Person-to-person transmission has also been described [9].The poultry slaughtered and dressed under open Kolkata market conditions carrying high initial contamination would be exhibited to the point the consumers are offered as retail meat. So, retail meat would harbour all the bacteria that are already present in meat as inherent contamination through infection and that are introduced during handling, improper dressing, cleaning, insanitary condition and retailing. To increase meat quality, assurance in accordance with microbial load assessment is deemed necessary. Hence, this study was conducted to assess the microbiological situation of fresh chicken and mutton meat which can be the reflection of hygienic condition of meat consumed and the possible hazards of public health.

II. Materials and Methods

Meat samples for the study were collected from different open meat markets or outlets of Kolkata. Most of the samples were collected from road side meat shop. In this study different parts of chicken like thigh, breast and wings and in mutton thigh, neck and groin were considered randomly as source of inoculums. A total of 200 samples, Chicken 100 and Mutton 100 were collected from different open markets in and around Kolkata. The samples were usually collected in the morning hours and in cool conditions in a sterile wide-mouthed container and preserved and transported in ice-packed thermocol bag. Meat samples amounting to 20 grams (approximately) were collected in sterile containers having normal saline and transported to the laboratory at a temperature of about 4 to 8°C. Macroscopic examination included physical appearance of the meat samples to look for any gross pathological lesions, checking for any blood, fresh or clotted, on the sample surface and also to detect fecal contamination of the samples, if any. Basically the meat samples were collected from 5 different areas of Kolkata- Central, East, West, North and South. With the help of sterile scissors, about 5 gm of meat samples were collected in sterile containers. 5 ml of sterile Normal Saline was added to it. The samples were then cut into smaller pieces under a biological safety cabinet. Grinding of the whole sample mixture was done in a mortar and pestle and finally the mixture was transferred in a sterile large test tube and shaken vigorously in a vortex mixture until a smooth emulsion of the sample was obtained. About 1 loopful of diluted inoculum was directly streaked in Nutrient Agar (NA), MacConkey Agar (MAC), Deoxycholate Citrate Agar (DCA) and Thiosulfate-citrate-bile salts-sucrose Agar (TCBS) plates. These samples were then incubated at 37°C for 24h, observed and recorded. Again about 1 loopful of prepared inoculum was inoculated in test tube containing 10 ml of Selenite Broth. The Selenite broth inoculated with sample was incubated at 37°C for 24h enrichment of *Salmonella spp.*

III. Results and Discussions

The General Viable Count (GVC) was done by performing serial dilution of the sample (10% w/v) in 0.1% peptone water and plated on plate count agar. For Total Enterobacteriaceae Count (TEC), 11 g of the sample was inoculated in 99 ml of buffered peptone water (0.1% peptone+0.85% NaCl) and lactose broth containing glass beads, respectively and incubated in shaker for 24 hours [10]. TEC was done by plating the serially diluted enriched sample on Violet Red Bile Glucose Agar (VRBGA). Incubation of GVC and TEC were done aerobically at 37°C for 24 h. The result of the present study indicated the presence of high microbial load of GVC and TEC from various parts of meat samples. Chicken Wings showed highest bacterial load of GVC and TEC, same way groin part of mutton showed highest bacterial load of GVC and TEC (Table 1).

In this study a variety of microorganisms had been isolated from the meat samples which are pathogenic as well as nonpathogenic in nature.

Pathogenic bacteria contribute to other globally important diseases, such as Pneumonia, which can be caused by bacteria such as Streptococcus and Pseudomonas, and foodborne illnesses which can be caused by bacteria such as Shigella and Salmonella.

Staphylococcus or Streptococcus are conditionally pathogenic and are also part of the normal human flora, they usually exist on the skin or in the nose without causing diseases.

Pseudomonas aeruginosa are basically opportunistic pathogens, but they are inheritingly Multi drug resistant and also resistant to common antiseptics. These bacteria can cause disease mainly in people suffering from immunosuppresion.

In this study, the pathogenic microorganisms were isolated in different percentages viz. *E. coli* (98%), *Enterococcus faecalis* (90%), *Staphylocoocus aureus* (20%), *Staphylococcus epidermidis* (20%), *Pseudomonas spp.* (10%), *Salmonella spp.* (2%), *Bordetella spp.* (1%). Other organisms that were isolated in this study were *Klebsiella pneumoniae* (98%), *Enterococcus aerogenes* (90%), *Micrococcus spp.* (69%), *Citrobactor spp.* (52%), *Proteus spp.* (50%), *Klebsiella oxytoca* (35%) and *Providencia spp.* (22%). (Table 2).

It was found that *Enterococcus faecalis* and *Enterobacter aerogenes* were present in 90% of the samples. Study reported the prevalence of *E. faecalis* in raw chicken meat of Japan [11]. Isolation of *E. faecalis* from raw chicken meat has also been reported by [12]. The results obtained showed that *E. coli* is more prevalent in the samples than *Salmonella sp.* This is in conformity with a study, who also reported high prevalence of *E. coli* than *Salmonella sp.* [8]. High prevalence of *E. coli* in retail meat market had also been reported [13]. The presence of *E. coli* strains in meat and meat products have been studied by many researchers [14]. Present findings also revealed the presence of *Salmonella sp.* which can be supported by the report of study separately reported contamination levels of this isolate in retail foods [15]. Prevalence of Salmonella sp. in various raw meat samples of local market had also reported by another study which supports our study [16]. *Klebsiella pneumoniae* showed 98% prevalence in the samples, which is in consonance with the findings of study revealed the prevalence and emergence of multidrug resistant *Klebsiella pneumoniae* in chicken [17]. Raw meat remains an important and probably the major source of human food borne infection with pathogenic bacteria. In spite of decades of effort it has been difficult to obtain food animals free of pathogenic bacteria.

Meat and poultry carcasses and their parts are frequently contaminated with pathogens, which reach the carcasses from the intestinal tract or from faecal material on feet and feathers. Cross contamination is a particular problem and several recommendations have been published to control pathogens throughout the chain from hatcheries to the preparation in the home. Certain animal illnesses may lead to a higher probability of mistakes in the processing plant, such as gastrointestinal ruptures, which would lead to increased microbial contamination and cross-contamination [18]

In Kolkata, meat is highly consumed animal originated food item. With high nutritive value, having both essential macro- and micronutrients, chicken meat makes an important part of a balanced diet for local people of Kolkata. Retailed meat and meat products are normally sold in markets in unhygienic conditions. There are no certified meat processing units and the hygiene in retail markets environment is much below the required standards. Therefore, the objective of the present study was to analyze the retailed meat samples of Kolkata for the presence of microorganisms, with special emphasis on isolation and identification of pathogenic contaminants.

IV. Conclusion

The presence of bacteria in meat has been widely reported from different parts of the world [19]. Most of the pathogens that play a role in food borne diseases have a zoonotic origin [20]. It can be concluded that the organisms, like *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* were the most prevalent microorganisms in the meat samples [21]. These contaminants not only possess health hazards to indigenous consumers but also to visitors exposed to consumption of such meats. Though not in the same concentration, these are important microbial contaminants in the retail chicken meats, which need to be taken care of ,for prevention of health hazards of the consumers by adopting proper sanitation, storage and retail practices. The markets from where the meat collected were mostly unhygienic. No proper drainage systems were present in most of the areas. Most of the markets were open markets with no proper *pucca* shops. No proper cold storage systems were available in the markets. These conditions provided favorable environment for cross-contamination, growth and multiplication of different microorganisms including the pathogenic ones, considering the suitable temperature, humidity and supply of nutrients from the meat samples themselves. This has a definite implication from the Public Health point of view. The findings indicate substantial presence of microbial contaminants in chicken and mutton meat samples in open markets of Kolkata where dearth of proper sanitation in the market places prevails.

References

[1]. Komba E. V. G., Komba, E. M. Mkupasi, A. O. Mbyuzi, S. Mshamu, D. Luwumbra, Z. Busagwe and A. Mzula, Sanitary practices and occurrence of zoonotic conditions in cattle at slaughter in Morogoro Municipality, Tanzania: implications for public health. Tanzania Journal Health Research, 14 (2), 2012.

[2]. Ercolini. D, F. Russo, E. Torrieri, P. Masi and F. Villani. Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. Applied and Environmental Microbiology.72 (7), 2006, 4663-4671.

[3]. Li M. Y., G. H. Zhou, X. L. Xu, C. B. Li and W. Y. Zhu. Changes of bacterial diversity and main flora in chilled pork during storage using PCR- DGGE. Food Microbiology. 23 (7), 2006, 607-611.

[4]. Adu-Gyamfi A., W. Torgby-Tetteh and V. Appiah . Microbiological Quality of Chicken Sold in Accra and Determination of D10-Value of E. coli. Food and Nutrition Sciences. 3 (5), 2012, 693-698.

[5]. Nørrung B., J. K. Andersen and S. Buncic. Main Concerns of Pathogenic Microorganisms in Meat Safety of Meat and Processed Meat. F. Toldrá, ed. Food Microbiology and Food Safety. (Springer New York), 2009, pp. 3-29.

[6]. Petersen, K.E. and W.O. James. Agents, vehicles, and causal inference in bacterial foodborne disease outbreaks: 82 reports (1986-1995). Journal of American Veterinary Medical Association. 212 (12), 1998, 1874-1881.

[7]. Tood, E.C. Epidemiology of foodborne diseases. World Health Stat. Q., 50(1-2), 1997, 30-50.

[8]. Zhao, C., B. Ge, J.D. Villena, R. Sudler and E. Yeh et al., 2001. Prevalence of Campylobacter spp., Escherichia coli and Salmonella serovars in retail chicken, Turkey, pork and beef from the Greater Washington D.C., Area. Applied and Environmental Microbiology, 67(12), 2001, 5431-5436.

[9]. Tauxe, R.V. Emerging foodborne diseases: An evolving public health challenge. Emerging Infectious Disease. 3(4), 1997, 425-434.

[10]. Forward, K.R., K.M. Matheson, M. Hiltz, H. Musgrave and C. Poppe. Recovery of cephalosporin-resistant Escherichia coli and Salmonella from pork, beef and chicken in Nova Scotia. Canadian Journal of Infectious Diseases and Medical Microbiology, 15(4), 2004, 226-230.

[11]. Tanimoto, K., T. Nomura, H. Hamatani, Y.H. Xiao and Y. Ike. A vancomycin-dependent VanA-type Enterococcus faecalis strain isolated in Japan from chicken imported from China. Letters in Applied Microbiology, 41(2), 2005, 157-162.

[12]. Simjee, S., D.G. White, D.D. Wagner, J. Meng, S. Qaiyumi, S. Zhao and P.F. McDermott. Identification of vat (E) in Enterococcus faecalis isolates from retail poultry and its transferability to Enterococcus faecium. Antimicrobial Agents and Chemotherapy, 46(12), 2002, 3823-3828.

[13]. Kumar, H.S., S. Ottu, I. Karunasagar and I. Karunasagar. Detection of Shiga-toxigenic Escherichia coli (STEC) in fresh seafood and meat marketed in Mangalore, India by PCR. Letters in Applied Microbiology, 33(5), 2001, 334-338.

[14]. Dutta S., A. Deb, U. K. Chattopadhyay and T. Tsukamoto . Isolation of Shiga toxin-producing Escherichia coli including O157:H7 strains from dairy cattle and beef samples marketed in Calcutta, India. Journal of Medical Microbiology. 49 (8), 2000, 765-767.

[15]. Soultos N, Koidis P, and Madden RH. Presence of Listeria and Salmonella spp. in retail chicken in Northern Ireland. Letters in Applied Microbiology, 37(5), 2003, 421–423.

[16]. Maharjan, M. Joshi, V. Joshi, D.D. Manandhar, P. Prevalence of Salmonella Species in Various Raw Meat Samples of a Local Market in Kathmandu. Annals of the New York academy of sciences. Volume 1081, 2006, 249-256.

[17]. Kim, S.H., C.I. Wei, Y.M. Tzou and H. An. Multidrug-resistant Klebsiella pneumoniae isolated from farm environments and retail products in Oklahoma. Journal of Food Protection. 68 (10), 2005, 2022-2029.
[18]. Singer, R.S., L.A. Cox, J.S. Dickson, H.S. Hurd, I. Phillips and G.Y. Miller. Modeling the relationship between food animal health and human foodborne illness. Preventive Veterinary Medicine. 79, 2007, 186-203.
[19]. Holds, G., A. Pointon, M. Lorimer, A. Kiermeier, G. Raven, and J. Sumner. Microbial profiles of carcasses and minced meat from Kangaroos processed in South Australia. International Journal of Food Microbiology. 123, 2007, 88-92.
[20]. Busani, L., G. Scavia, I. Luzzi and A. Caprioli, 2006. Laboratory surveillance for prevention and control of foodborne zoonoses. Annali dell'Istituto Superiore di Sanità. 42(4), 2006, 401-404.
[21]. Saikia, P. and S.R. Joshi. Retail Market Poultry Meats of North-East India-A Microbiological Survey for Pathogenic Contaminants. Research Journal of Microbiology. 5(1), 2010, 36-43.

Table 1 (a) (b): Microbial load of GVC and TEC of raw meat samples (Chicken & Mutton)

Meat parts of chicken	GVC(cfu/g)	TEC (cfu/g)
Thigh	2.4×10^6	2×10^4
Breast	3.8×10^6	2×10^4
Wings	4.1×10^6	2.25×10^5

Meat parts of Mutton	GVC (cfu/g)	TEC (cfu/g)
Thigh	2.1×10^6	1.6×10^5
Groin	1.1×10^7	1.7×10^5
Neck	2×10^6	1.25×10^5

Table 2: Prevalence of contaminant microbes in the raw meat sample (Chicken & Mutton)

Isolates	Positive Percentage (%)	Chicken sample (%)	Mutton sample (%)
Escherichia coli	98	100	96
Klebsiella pneumoniae	98	100	96
Enterococcus faecalis	90	93	87
Enterobacter aerogenes	90	95	85
Micrococcus spp.	69	72	66
Citrobacter spp.	52	60	44
Proteus spp.	50	52	48
Klebsiella oxytoca	35	35	35
Providencia spp.	22	21	23
Staphylococcus aureus	20	22	18
Staphylococcus epidermidis	20	17	23
Pseudomonas spp.	10	9	11
Salmonella spp.	2	2	2