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Retail Market Poultry Meats of North-East India-A Microbiological Survey for Pathogenic Contaminants

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Abstract: A cross-sectional study of different portions of chicken raw meat samples from the local meat markets of North East India was carried out during October 2007 to September 2008 on a total of 110 collected samples using Plate Count Agar, Lactose broth, Violet red bile glucose agar, Brilliant green bile lactose broth, Eosin methylene blue agar, MacConkey agar, Salmonella shigella agar, Bimuth sulphite agar, Xylose lysine deoxycholate agar, Nutrient agar and Potato dextrose agar. Of the 74 different bacteria detected, the bacterial population incidence was highest in chicken wings (83.5%), followed by chicken tails (77%), breasts (70%), thighs (69%) and gizzard (38%). Frequent organisms in the samples were *Enterococcus faecalis* (100%), *Enterobacter aerogenes* (100%), *Escherichia coli* (98%), *Klebsiella pneumoniae* (98%), *Micrococcus* sp. (69%) and *Candida* sp. (80%). The other organisms isolated were *Klebsiella oxytoca* (35%), *Citrobacter* sp. (52%), *Proteus* sp. (49%), *Staphylococcus aureus* (20%), *Staphylococcus epidermidis* (20%), *Yersinia enterocolitica* (23%), *Listeria monocytogenes* (15%), *Shigella dysenteriae* (1.8%), *Salmonella tyhpi* (20%), *Bacillus cereus* (10%), *Aeromonas* sp. (5.5%), *Alcaligens faecalis* (15%), *Penicillium* sp. (42%), *Aspergillus* sp. (20%) and *Rhodotorula* sp. (5.5%). This finding indicates substantial presence of microbial contaminants in retail chicken meat samples in North East India and dearth of proper sanitation in the market places.

Key words: Chicken meat, local markets, sanitation, microbial, contaminants

INTRODUCTION

Microbial food safety and food-borne infections are important public health concern worldwide. There have been a number of food-borne illnesses resulting from the ingestion of contaminated foods such as chicken meats. Most of the pathogens that play a role in foodborne diseases have a zoonotic origin (Busani *et al*., 2006). According to reports of the World Health Organization (WHO, 2003) and the Centers for Disease Control and Prevention (CDC, 2000) every year a large number of people are affected by diseases due to contaminated food consumption. Although, these pathogens usually cause mild to moderate self-limiting gastroenteritis, invasive diseases and complications may occur, resulting in more severe cases (Zhao *et al*., 2001). Numerous epidemiological reports have implicated foods of animal origin as the major vehicles associated with illnesses caused by food-borne pathogens (Petersen and James, 1998; Tood, 1997). Contaminated raw or undercooked poultry and red meats are particularly important in transmitting these food-borne pathogens (Zhao *et al*., 2001). Person-to-person transmission has also been described (Tauxe, 1997).

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Pathogens like *Campylobacter*, *Salmonella* and pathogenic *E. coli* all colonize the gastrointestinal tracts of a wide range of wild and domestic animals, especially animals raised for human consumption (Meng and Doyle, 1998). Studies worldwide have shown that such pathogens are often present in fresh meat and poultry (Tood, 1997). The health status of food animals can potentially influence food borne pathogen levels in three ways. First, diseased animals may shed higher levels of food borne pathogens. Second, animals that require further handling in the processing plant to remove affected parts may lead to increased microbial contamination and cross-contamination. Finally, 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 (Singer *et al.*, 2007).

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 (Dinçer and Baysa, 2004).

North-East India is inhibited by different ethnic tribes, where chicken meat is one of the 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 these people. Retail 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 North-East India for the presence of microorganisms, with special emphasis on isolation and identification of pathogenic contaminants.

MATERIALS AND METHODS

Sample Collection

Meat samples were collected randomly from October 2007 to September 2008 in sterilized container from different markets spread over seven states of North-East India and were kept at 4°C until further analysis. Different parts of chicken meat like thighs, breasts, gizzards, tails, livers, wings and hearts were considered as source of inoculums for the study.

Microbial Analysis

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) and Total Coliform Count (TCC), 11 g of the sample was inoculated in 99 mL of buffered peptone water (0.1% peptone+0.85% NaCl) and lactose broth (Forward *et al.*, 2004) containing glass beads, respectively and incubated in shaker for 24 h (Aneja, 2005). TEC was done by plating the serially diluted enriched sample on Violet Red Bile Glucose Agar (VRBGA) and TCC by MPN technique using Brilliant Green Bile Lactose Broth (BGLB). Incubation of GVC, TEC and TCC were done aerobically at 37°C for 24 h. Isolation of *E. coli* was done by direct inoculation of the enriched sample from Lactose broth on EMB agar and for other microorganisms it was done by inoculating the isolates obtained from GVC and TEC on Nutrient Agar. Lactose fermenters and non lactose fermenters were detected by inoculating enriched (in buffered peptone water) sample into MacConkey agar plate and incubated for 24 h at 37°C. For selective isolation of *Salmonella* enriched

(in buffered peptone water) sample was streaked into Bismuth Sulphite Agar (BSA) and subcultured into Xylose Lysine Deoxycholate (XLD) agar and Salmonella-Shigella (SS) agar plates. The inoculated plates were incubated for 48 h at 37°C. Yeast and mold count were done on Potato Dextrose Agar (PDA) by serial dilution method and incubated at 25°C for 7 days.

Identification of the Isolates

The bacterial isolates, obtained from the samples were purified by re-streaking them on the media used for their isolation and were characterized by different biochemical tests. The biochemically characterized isolates were identified with the help of Bergeys Manual of Determinative Bacteriology (Holt *et al.*, 1994). Identification of the mold and yeast were done by staining and sugar assimilation tests, respectively.

RESULTS

Microbial Analysis

The average microbial load of GVC, TEC and TCC and isolated from various parts of chicken meat collected from the North Eastern states were listed in Table 1. Tail and wings revealed higher general viable counts, which were 1.8×10^7 cfu g^{-1} and 4.1×10^7 cfu g^{-1}, respectively compared to other parts while gizzard showed consistently lower counts (5.0×10^5 cfu g^{-1}). Total enterobacteriacae count was found to high in tail portion (1.6×10^5) cfu g^{-1}. Total coliform count was found to be higher in tail and wing portion. It showed MPN index of 0.7.

Yeast and mould was not detected in gizzard portion, whereas high count was detected again in wing portion.

Assam, Manipur and Meghalaya showed high general viable counts, whereas the other three states showed comparatively low general population count (Table 2).

Identification of the Isolates

The bacterial isolates were identified based on their morphology and biochemical characters. Biochemical test results of bacterial isolates were listed in Table 3. However, some

Table 1: Microbial load of GVC, TEC, TCC and yeast and mould count of raw meat samples

Chicken meat parts	General viable count (cfu g^{-1})	Total entero-bacteriaceae count (cfu g^{-1})	Total coliform count (TCC) MPN index	Yeast and mold count (cfu g^{-1})
Thighs	2.4×10^6	2.0×10^4	2.5	1.2×10^4
Breasts	3.7×10^6	2.0×10^4	0.4	1.3×10^4
Gizzard	5.0×10^5	2.3×10^3	0.3	ND
Tail	1.8×10^7	1.6×10^5	0.7	1.0×10^5
Liver	3.0×10^6	1.0×10^4	0.3	1.3×10^4
Wings	4.1×10^7	1.2×10^5	0.7	1.3×10^5
Heart	2.4×10^6	2.3×10^4	0.3	1.3×10^4

ND: Not detected

Table 2: Average microbial count detected from various sampling sites

States of North-East India	General viable count (cfu g^{-1})	Total entero-bacteriaceae count (cfu g^{-1})	total Coliform count (TCC) MPN index	Yeast and mold count (cfu g^{-1})
Arunachal Pradesh	2.1×10^6	1.6×10^5	0.3	1.0×10^4
Assam	1.0×10^7	2.0×10^5	2.5	1.3×10^3
Manipur	1.1×10^7	1.3×10^5	0.4	1.0×10^2
Meghalaya	1.0×10^7	1.0×10^5	0.7	2.7×10^4
Mizorum	2.0×10^6	2.0×10^4	0.3	2.0×10^3
Nagaland	2.0×10^6	1.2×10^5	0.7	1.0×10^4
Tripura	3.0×10^6	1.7×10^5	0.4	1.0×10^3

Table 3: Biochemical results of the identified isolates from chicken meat sample

Sl. No	Gram's reaction	Oxidase test	Catalase test	Indole test	MR test	VP test	Citrate use	Urease test	TS test	Nitrate reduction	Starch hydrolysis	Sugar utilization test							Identified organisms
												Glucose	Lactose	Sucrose	Mannitol	Arabinos	Sorbitol	Maltose	
1	+ve cocci	-	+	-	+	-	-	-	ND	+	-	A	-	ND	AG	ND	ND	ND	*Staphylococcus aureus*
2	+ve cocci	-	+	-	-	-	-	+	ND	-	-	A	A	A	-	-	-	A	*Staphylococcus epidermidis*
3	Rod+	+	-	-	-	-	-	-	ND	+	+	A	-	A	-	-	ND	ND	*Bacillus cereus*
4	+ve cocci	+	+	-	-	-	-	+	ND	+	+	A	-	A	-	-	-	A	*Micrococcus* sp.
5	-ve rod	-	+	+	+	-	-	-	A/A,G+, H_2S-	+	-	AG	AG	-	AG	-	-	AG	*Escherichia coli*
6	-ve rod	-	+	-	-	+	+	+	A/A, G+, H_2S-	+	-	AG	AG	AG	AG	ND	ND	ND	*Klebsiella pneumoniae*
7	-ve rod	-	+	+	+	-	+	+	A/A, G-, H_2S-	-	-	A	AG	A	AG	A	A	A	*Klebsiella oxytoca*
8	-ve rod	-	+	-	+	-	+	-	H2S+	+	-	A	-	-	AG	-	ND	-	*Salmonella tyhpi*
9	-ve rod	-	+	-	-	-	-	-	A/K, G-, H_2S-	+	-	A	-	-	-	-	-	-	*Shigella dysenteriae*
10	-ve rod	-	+	-	-	+	+	-	A/A,G+, H_2S-	+	-	A	AG	A	AG	-	ND	ND	*Enterobacter aerogenes*
11	-ve rod	-	+	-	+	-	+	+	A/A,G+, H_2S+	+	-	AG	-	-	-	ND	ND	A	*Proteus* sp.
12	+ve rod	-	+	-	+	+	-	-	A/K, G-, H_2S	ND	-	A	-	-	-	ND	ND	ND	*Listeria monocytogenes*
13	-ve rod	+	+	-	+	+	-	-	A/AG-, H_2S-	ND	+	A	A	-	A	ND	A	ND	*Aeromonas* sp.
14	-ve rod	+	+	-	-	-	-	-	ND	-	-	-	-	-	-	-	-	-	*Alcaligens faecalis*
15	+ve rod	+	-	ND	ND	ND	ND	-	ND	ND	+	A	A	A	A	-	A	A	*Enterococcus* faecalis
16	-ve rod	-	+	-	+	-	+	-	A/A, G+ H_2S+	ND	-	A	-	ND	-	ND	ND	ND	*Citrobacter* sp.
17	-ve rod	+	-	-	-	-	-	+	A/A, G- H_2S-	ND	-	A	-	A	ND	-	-	ND	*Yersinia enterocolitica*

+: Positive, -: Negative, A/A,G- H_2S-: Acid butt acid slant gas (-) ve H_2S (-)ve, A: Acid production, MR: Methyl red, A/K G-, H_2S-: Acid butt alkaline slant gas (-) ve H_2S (-) ve, AG: acid and gas production, VP: Voges proskauer, A/A G+, H_2S+: Acid butt acid slant Gas (+) ve, H_2S (+) ve, ND: Not detected, TSI: Triple sugar iron

isolates need further tests to classify them into species level. Fungal population were identified based on their morphology and micrograph and were identified as *Penicillium* sp. and *Aspergillus* sp. Yeast isolates were identified as *Candida* sp. and *Rhodotorula* sp. depending on their morphology and sugar assimilation tests.

Out of the 110 raw meat samples studies for the microbial load, *Enterococcus faecalis* and *Enterobacter aerogenes* showed 100% positive result while *Escherichia coli* and *Klebsiella pneumoniae* showed 98% prevalence. The frequency of other isolates were *Klebsiella oxytoca* (35%), *Citrobacter* sp. (52%), *Proteus* sp. (49%) and *Micrococcus* sp. (69%). Less frequently isolated microbes were *Staphylococcus aureus* (20%), *Staphylococcus epidermidis* (20%), *Yersinia enterocolitica* (23%), *Listeria monocytogenes* (15%), *Shigella dysenteriae* (1.8%), *Salmonella* sp. (12.37%), *Aeromonas* sp. (5.5%), *Alcaligens* faecalis (15%) and *Bacillus cereus* (10%). Molds and yeast isolated were *Penicillium* sp. (42%), *Aspergillus* sp. (20%) and *Candida* sp. (80%), *Rhodotorula* sp. (5.5%) (Table 4).

Escherichia coli is more prevalent in the samples than *Salmonella* sp. in different portion of the meat samples analyzed (Table 5). *Escherichia coli* showed 98.14% prevalence, whereas *Salmonella* sp. was found to be only 12.37%.

Table 4: Prevalence of contaminant microbes in the sample

Isolates	Positive cases out of 110 samples	Percentage (%)
Enterococcus faecalis	110	100
Enterobacter aerogenes	110	100
Escherichia coli	108	98
Klebsiella pneumoniae	108	98
Klebsiella oxytoca	39	35
Citrobacter sp.	57	52
Proteus sp.	54	49
Micrococcus sp.	76	69
Staphylococcus aureus	22	20
Staphylococcus epidermidis	22	20
Yersinia enterocolitica	25	23
Listeria monocytogenes	16	15
Shigella dysenteriae	2	1.8
Salmonella sp.	22	20
Aeromonas sp.	6	5.5
Alcaligens faecalis	16	15
Bacillus cereus	11	10
Penicillium sp.	46	42
Aspergillus sp.	22	20
Candida sp.	88	80
Rhodotorula sp.	6	5.5

Table 5: Prevalence of *E. coli* and *Salmonella* sp. in the chicken meat samples

		Primary culture										
		Growth in EMB agar		Growth in MacConkey agar: Lac. fer.		Growth in MacConkey agar: Non lac. fer.		Sub-culture				
Part of meat Sample	Total sample processed	+ve	%	+ve	%	+ve	%	Growth in BSA+ve	Growth in SS+ve	Growth in XLD+ve	Total +ve	Total %
Thighs	16	16	100	16	100	5	31	2	2	1	2	12.5
Breasts	16	16	100	16	100	4	25	2	1	1	1	6
Gizzard	16	15	94	16	100	0	-	0	0	0	0	-
Tail	16	16	100	16	100	9	56	4	4	4	4	25
Liver	15	15	100	15	100	6	37.5	1	1	1	1	6
Wings	16	16	100	16	100	0	-	0	0	0	0	-
Heart	15	14	93	15	100	0	-	0	0	0	0	-

Lac. fer: Lactose fermenter Non lac. fer: Non-lactose fermenter, EMB: Eosin Methylene Blue agar, BSA: Bismuth Sulfite agar, SS: *Salmonella-Shigella* agar, XLD: Xylose Lysine Deoxycholate agar

DISCUSSION

Chicken meat is an important source of protein and a valuable commodity in resource limited tribal communities, such as in North-East India. New problems of food borne zoonotic diseases are arising in conjunction with the development of different types of food of animal origin. There are a number of factors responsible for the spread of zoonotic disease such as pathogenesis related to contaminated food of animal origin. The present study evaluates that most of the retail shops do not operate in a safe and sanitized environment. Covering and hanging carcasses were rarely observed. Further, the chopping blocks of wood were found to be same everyday without proper cleanliness. This enhanced the chances of cross contamination of uninfected carcass if any prior carcass happens to be infected. The processing of carcass surface into parts further spreads contamination by exposing more carcass surface and susceptible fleshy parts to the contaminants if the same cutting blocks and knives are used (Satin, 2002).

Wide ranges of microorganisms were found to be associated with the meat samples. Bacterial count was comparatively higher than yeast and fungal count. It is expected because meat products are found to be kept in the open environment, the temperature that allows the bacteria to grow in a good growth rate than yeast or fungi. The result of the present study indicated the presence of high microbial load of GVC, TEC, TCC (MPN index) and yeast and mould count isolated from various parts of chicken meat. Among the various chicken meat parts wings showed highest bacterial load of GVC and TEC. In this study, Assam, Mizoram and Nagaland showed high general viable counts, whereas the other three states showed comparatively low general population count. This may be due to the comparatively higher ambient temperature prevalent in these areas as high temperature favours good growth of the microbes. It was found that *Enterococcus faecalis* and *Enterobacter aerogenes* were present in all the samples. Tanimoto *et al.* (2005) reported the prevalence of *E. faecalis* in raw chicken meat of Japan. Isolation of *E. faecalis* from raw chicken meat has also been reported by Simjee *et al.* (2002). The results obtained showed that *E. coli* is more prevalent in the samples than *Salmonella* sp. This is in conformity with Zhao *et al.* (2001) who also reported high prevalence of *E. coli* than *Salmonella* sp. High prevalence of *E. coli* in retail meat market had also been reported by Kumar *et al.* (2001). Present findings also revealed the presence of *Listeria* sp. and *Salmonella* sp. which can be supported by the report of Soultos *et al.* (2003) and Inoue *et al.* (2000) who separately reported contamination levels of these isolates in retail foods. Prevalence of *Salmonella* sp. in various raw meat samples of local market had also reported by Maharjan *et al.* (2006) which supports our study. *Klebsiella pneumoniae* showed 98% prevalence in the samples. This is in consonance with the finding of Kim *et al.* (2005) revealed the prevalence and emergence of multidrug resistant *Klebsiella pneumoniae* in chicken.

Yeast and mould population were isolated in low frequency compared to the bacterial population. Among the yeast isolates, *Candida* sp. showed 80% prevalence and among the moulds, *Penicillium* sp. showed 42% prevalence. There was no contamination by yeasts and moulds in gizzard. Although, *Aspergillus* sp. was found to have 20% incidence, this may be good enough to produce toxins based on its prevalence and production of food toxins are well reported in species of *Aspergillus*. Altalhi and Albashan (2004) had also reported contamination of chicken meat with *Penicillium* sp. and *Aspergillus* sp. The finding that the presence of *Candida* sp. and *Rhodotorula* sp. in meat is similar to record of Viljoen *et al.* (1998) who outlined the presence of yeast population associated with poultry carcasses.

It can be concluded that the organisms like *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Penicillium* sp. *Aspergillus* sp. *Candida* sp. and *Rhodotorula* sp. were the most prevalent microorganisms in the meat samples collected during the study from various market places of North-East India. 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.

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