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Causes, Concerns, Consequences and Control of Microbial Contaminants in Meat-A Review

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ABSTRACT

The aim of this study was to evaluate status of microbial contaminants in food of animal origin. Emergence and re-emergence of diseases due to pathogenic bacteria are the key issue of the new pattern of food trades. Food poisoning or food intoxication syndrome is a global problem for meat industry. The bacterial pathogens most frequently identified from illness associated with beef products are *Salmonella* sp., *Campylobacter*, *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Clostridium perfringens*, *Yersinia enterocolitica*, *Bacillus cereus* and *Vibrio parahaemolyticus*. Microbial contaminants rather common than any other form of contaminants as food animals itself harbour them hence, microbial contamination of carcass surfaces is unavoidable. Most of the micro floras transferred to the carcasses are nonpathogenic, but some pathogens like *Salmonella* sp., *Escherichia coli* O157:H7, *Campylobacter* sp. and *L. monocytogenes* may be present and poses a safety challenge to the meat industry. Novel methods such as immunological, chemical, biochemical, biophysical, nucleic acid probe, Polymerase Chain Reaction (PCR) and more recently biosensor based techniques have been developed to monitor the incidence of pathogenic bacteria in meat foods. In recent years, increase in global trade and awareness of the consumers about the hygienic quality of the meat, international attention is being focused on ways to improve the microbial quality and safety of meat foods. The present review confirmed the importance of maintaining good process hygiene at meat packing plants for further improvement of microbiological status of meat.

Key words: Meat, microbial contaminants, pathogens, polymerase chain reaction, food poisoning, meat quality

INTRODUCTION

The office of the United States Trade Representative has estimated that international trade has increased fivefold since, signing of General Agreement on Tariff and Trade (GATT) in 1947. The formation of World Trade Organization (WTO) in 1995 resulted in significantly increased trade in foods of animal origin and live animals between different countries. But emergence and re-emergence of diseases due to pathogenic bacteria are the key issue of the new pattern of meat

food trades. According to CDCP (1998) report, annual cost due to foodborne illness in the United States is nearly 10 billion US\$. The bacterial pathogens most frequently identified from illness associated with beef products are *Salmonella* sp., *Clostridium perfringens* and *Staphylococcus aureus*. Interest on *Escherichia coli* O157: H7 has increased after highly publicized outbreak of food poisoning associated with undercooked beef patties in the United States in 1993 though it was confined to North America until mid 1990s. Likewise, multidrug resistant *Salmonella typhimurium* DT-104 spread widely since, they were first detected in United Kingdom (JECFA, 2002). The incidence of *Salmonella* was recorded up to 9% in red meat in India (Rao and Mahendrakar, 2003). These potential bacterial pathogens reside in hide or in intestinal tract of food producing animals or may be originating indirectly by cross contamination or through processing environment (Buckle *et al.*, 1989). Other foodborne emerging diseases include *Listeriosis*, which spread throughout the France and also in Canada, where meat and meat products were implicated as a source of *Listeria monocytogenes* (Borch and Arinder, 2002). Similarly, *Staphylococcal* food poisoning or food intoxication syndrome was first reported in 1894, it is now a global problem in meat industry. In this review, we have tried to present the scenario about meat borne pathogens, the major sources of contamination, their detection, incidences of pathogen contamination in meat foods, the public health risk and relevant regulations.

Foodborne pathogens: Pathogens are virtually inescapable, reaching every aspect of life. Potentially threatening bacteria in foods, soil and in water has historically outrun any detection efforts resulting in unwarranted deaths and illness. Current trends in nutrition and food technology are increasing the demands on food microbiologist to ensure a safe food supply. Microbial contaminants are extremely difficult to pinpoint precision of their presence and role in food systems. Available literature suggests that the evidence of foodborne spoilage and pathogenic bacteria reported up from pre-scientific era. Lot of developments has taken place in bacteriology in 1900s and scientists identified a range of bacteria and now have no limits. Among all the microbes, *Salmonella* and *Campylobacter* are the most serious foodborne pathogens. These two bacteria are causing as many as 4 million illnesses and 4000 deaths year⁻¹ in USA (Bennett and Berry, 1987). Other important foodborne pathogenic bacteria include *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Yersinia enterocolitica*, *Bacillus cereus*, *Escherichia coli* and *Vibrio parahaemolyticus*. Although, the same food borne pathogens are found in many other countries, risks are different because of geographical differences in the animals and vector reservoirs, cultural differences of food consumption habits and processing conditions (ERS, 1999).

Sources of microbial contaminants in meat: Microbial contaminants rather common than any other form of contaminants as food animals itself harbour them. Microbial status of fresh meat depends on animal rearing, transportation, slaughtering and cutting and packaging, besides hygiene and processing conditions of the slaughter plant. The natural surface flora of meat animals usually is not important as the contaminating microorganisms from their intestinal or respiratory tracts. However, hides, hooves and hair contain not only large numbers of microorganisms from soil, manure, feed and water but also important kinds of spoilage organisms. The skin of many meat animals may contain *Micrococci*, *Staphylococci* and *Streptococci*. *Staphylococci* on the skin or from the respiratory tract may find their way onto the carcass and then to the final raw product. The faeces and faecal contaminated products of animals can contain many enteric organisms including *Salmonella*. People working in meat processing plants also can act as vector of many foodborne

pathogenic bacteria, but this represents only little importance (Frazier and Westhoff, 1999). Monitoring of these emerging contaminants and strict implementation of surveillance contributes positive benefits to importing and exporting countries. These benefits are improved health and nutritional status, economic advantages through job creation and improved diplomatic relation between the countries concerned, but this relies on testing and other forms of inspection by either exporting or importing country or both.

Detection of microbial contaminants: Currently, so many techniques are available for enumeration and isolation of microbial contaminants in foods. But, techniques explained by American Public Health Association (APHA, 1984) and International Commission on Microbiological Specification for Foods (ICMSF, 1978) for enumeration and isolation of bacteria are widely acceptable.

However, several novel methods such as immunological, chemical, biochemical, biophysical, nucleic acid probe, Polymerase Chain Reaction (PCR) and more recently biosensor based techniques have been developed to monitor the incidence of pathogenic bacteria in foods including meat (Fung, 1995). Inherent problems associated with such techniques include difficulties in the recovery of bacterial species from meat and other foods, as some co-extractive materials comes at the time of enrichment in selective broth medium or even successful, they are time consuming to carry out and can significantly extend duration of the isolation and detection procedure (Duffy *et al.*, 1999). Inefficiencies in extraction of the target pathogens from the food matrix and poor separation from elements of the competitive micro flora, can lead to subsequent problems in the accurate detection and/or differentiation of target organisms. Thus, co-extractive materials can interfere with DNA hybridization test in PCR assay and immunoassay (Beumer and Brinkman, 1989). Furthermore, these methods require approval by any Governmental Organization or other agencies such as CAC, AOAC, APHA, ISO etc. Traditional cultural and serological methods play a utopian goal in this area of concern, though time consuming and labour intensive.

Traditional and standardized analysis of food for presence of bacteria relies on the enrichment and isolation of presumptive colonies on solid media, using approved diagnostic artificial media. The International Organization for Standardization (ISO) has elaborated several standards for the detection of important pathogenic bacteria by traditional method. For example, *Salmonella* (ISO 6579), *Listeria monocytogenes* (ISO 10560), thermo-tolerant *Campylobacter* (ISO 10272), *E. coli* O157 (ISO 16654) and *Staphylococcus* sp. (ISO 6888) were enumerated by several workers (Anonymous, 1999, 2001; Biswas *et al.*, 2008).

Incidences of contaminants in meat: The microbiological profile of meat products is one of the key criteria for determining quality and safety of fresh produce. Ideally, meat should be considered as wholesome when pathogen of concerns is absent or even present at lower number depending on their toxins/metabolites in per unit basis or food lot. Various researchers had reported microbial contaminants in meat (Gill, 1998; Vanderlinde *et al.*, 1998; Biswas *et al.*, 2008). Vanderlinde *et al.* (1998) did an extensive study on microbial quality of beef carcass meat from retail outlets as well as export markets. In a similar study, Biswas *et al.* (2008) reported that buffalo meat from Indian meat packing plant contain comparatively less number of microbes than many developed and developing country. The log mean of SPC for frozen buffalo meat trimmings and silver sides were 4.18 and 2.98 g⁻¹. In other study, Ziauddin *et al.* (1994) reported that the differences in bacterial counts on the different regions of the carcasses as well as two slaughter units were marginal. The

SPC of leg, loin, shoulder and neck cuts varies from 4.82-4.92, 4.71-5.13, 5.41-5.49 and 4.52-4.80 log₁₀ cfu cm⁻², respectively. In surveys of seven European abattoirs, Roberts *et al.* (1984) reported that mean aerobic plate counts for beef carcasses ranged between 2.29 and 3.85 log units cm⁻². Beef carcass from Germany (Ingram and Roberts, 1976), New Zealand (Keeley, 1988) and USA (McNamara, 1995) showed average APC of 4.51, 4.51 and 2.68 log cfu cm⁻², respectively. However, trimmings had higher APC than different beef cuts (Scanga *et al.*, 2000).

Streptococcal species are faecal origin and are better indicators of food sanitary quality, especially for frozen foods. Gill (1998) reported that potential meat contamination of *Streptococcus faecalis* occurred during slaughtering and butchering of food animals. He further revealed that knife trimming do not contribute to enhancing microbiological quality of dressed carcasses, except aesthetic values. Chabela *et al.* (1999) elucidated that *Enterobacteriaceae* counts in beef meat were 10⁵ cfu g⁻¹. The incidence of coagulase-positive *Staphylococcus* sp., on both domestic and export beef carcasses in Australia were 20 and 29%, respectively (Vanderlinde *et al.*, 1998). But in US beef carcasses, the incidence was only 4.2% (McNamara, 1995). However, incidences of *L. monocytogenes* in meat vary widely from 0 to 92% and the contamination mostly occurred on the surface of meat and meat products (Farber and Peterkin, 1991). The organism may also thrived interior of muscle tissues of frozen beef. In a survey of 2089 steer/heifer carcasses in the USA, it has been revealed that incidence of *L. monocytogenes* was about 4.1%. However, no *L. monocytogenes* was reported in beef carcasses from Northern Ireland (Madden *et al.*, 2001). Though, in India, *L. monocytogenes* was first isolated from sheep, later several studies suggested the presence of this organism in buffalo meat (Chaudhari, 2001; Biswas, 2005; Biswas *et al.*, 2008).

The incidence of *Salmonella* sp., in food animals is wide. Foodborne outbreaks of salmonellosis associated with eating of beef have been reported by Roels *et al.* (1997). Abouzeed *et al.* (2002) have reported the prevalence of *Salmonella* sp., to be 4.6% in beef cattle on conducting examination of caecal contents in Canada. Patterson (1974) reported that the incidence of *Salmonella* sp., was 0.34% in Northern Ireland. Similarly, Vanderlinde *et al.* (1998) reported the incidence of *Salmonella* sp., in Australian frozen bulk packed meat was 0.22%. Likewise, Scanga *et al.* (2000) did a classical study for determination of level of contaminants in raw beef trimmings and ground meat. In their study, *Salmonella* sp., was found more frequently in fed-beef trimmings (5.2%) than culled beef cow trimmings (0%), culled dairy cow trimmings (0%) or imported trimmings. The incidences of *Salmonella* sp., in beef were also reported in the USA (Sofos *et al.*, 1999) and Mexico (Chabela *et al.*, 1999). Bachhil and Jaiswal (1988) recorded 5% of sample of fresh and frozen buffalo meat, 6.6% of minced meat and 10% Kabab were positive for *Salmonella* in India.

Escherichia coli, since its discovery by Theobald Escherich in 1885 has been receiving much greater importance due to its pathogenicity by certain strains both in man and animals. Worldwide contamination of this group of bacteria occurred in meat through soiling of the carcass and plant environment with faecal materials during slaughter process (Johnson *et al.*, 1996). The incidence of *E. coli* not very variable in domestic or export beef meat regardless the fat content in trimmings. The average *E. coli* Counts (ECC) in Indian buffalo meat were 1.1log cfu g⁻¹. In another study, Hazarika *et al.* (2005) screened 153 buffalo meat samples, among which 24.78% samples were positive for *E. coli*. However, enumeration data regarding various groups of *E. coli* is sparse.

Evidence of VTEC was found in 15 to 40% samples of ground or deboned raw beef in Canada (Acheson, 1996). Similarly, in the United Kingdom, 17% of raw beef samples contained VTEC. However, VTEC were found less frequently in continental Europe and only 1.8% of beef were

recorded positive (Pierard *et al.*, 1994). Elder *et al.* (1997) noted that among 28% of cattle presented for slaughter in Midwestern USA carried *E. coli* O157:H7, only 2% of carcasses sampled were positive. Other workers also reported incidences of *E. coli* (71% serotype O157) in Sweden (Anonymous, 2000).

Prevalence rate of verotoxic *E. coli* in meat and meat products has been recorded at an alarming rate in India. In a study, Rathore (2000) reported that 89.19% of 37 *E. coli* isolates were found verotoxic by vero-cell cytotoxicity assay. Similarly, *E. coli* strains isolated from different meat and meat products revealed 15.90% isolates to be verotoxigenic (Banerjee *et al.*, 2001). Hazarika *et al.* (2005) reported that 27% isolates of *E. coli* were verotoxigenic (VTEC) in vero-cell cytotoxicity assay. They further concluded that majority of VTEC isolates from meat and meat products of buffaloes were found positive for vt₂ gene (77.42%) followed by vt₁ (16.13%), while both vt₁ and vt₂ were detected only in 6.45% of the VTEC isolates.

Public health risk: There is considerable evidence of foodborne pathogens, mainly microbial origin, constitutes major health hazards. Among all the microbes, *Salmonella* and *Campylobacter* are the most serious foodborne pathogens. These two pathogens are causing as many as 4 million illness and 4000 deaths per year in USA (Bennett and Berry, 1987). The most common clinical manifestation of non-typhoid salmonellosis is that of acute gastro-enteritis with a short and shelf limiting clinical course. Bacteraemia may occur as a rare complication of any *Salmonella* infection and can degenerate into chronic condition such as osteomyelitis, cardiac inflammation or neural disorders. It has also been linked to one set of aseptic reactive arthritis and Reiter's syndrome. Severe infection occurs most often in the infant, elderly or immunocompromised patients. In the person infected with HIV, salmonellosis can be a severe invasive disease and recurrence of bacteraemic infection after appropriate therapy is common (Tauxe, 1991). Similarly, VTEC are associated with infant diarrhoea, hemorrhagic colitis, thrombotic-thrombolytic purpura and haemolytic uremic syndrome in human. However, *E. coli* O157:H7 is most common serotype isolated from individuals with haemorrhagic colitis.

Other important pathogenic bacteria associated with food safety issue is *Listeria* and coagulase positive *Staphylococcus*. Listeriosis can occur in healthy adults and children, however, the most vulnerable groups include pregnant women, infants, elderly and immunocompromised persons (Jaradat *et al.*, 2002). In pregnant women, the infection most commonly produces a flue like illness, complications often occur in the foetus and newborn, resulting in miscarriage, still birth or meningitis. In older children and adults, common symptoms are involvement of central nervous system, pneumonia endocarditis, localized abscess, skin lesions or conjunctivitis with high mortality rate (Miettinen *et al.*, 1999). However, *S. aureus* is also responsible for a variety of pyogenic skin diseases in man. This organism has also been associated with osteomyelitis, acute endocarditis, toxic shock syndrome, deep-seated abscesses in various muscle and organs and staphylococcal scald skin syndrome in newborn babies. Although, the same foodborne pathogens are found in many other countries, risks are different because geographical differences in the animals and vectors reservoirs, cultural differences of food consumption habits and of course processing conditions (ERS, 1999).

Regulations and international agencies: In contrast to chemical contaminants, for regulation of microbial contaminants, each country in the world have well controlled monitoring set-up for meat and meat products. But there is little apparent connection between public health goals and standards or guidelines except in general way of reducing or limiting contamination (Todd, 2003).

It is evident that no one country has microbial standard for all commercial foods. Several developed country like USA, UK, Germany, France, Italy and The Netherlands also come under same catalogue. In a recent comment it is explained, USDA and FDA also need to do a much better job on regulatory enforcement and they need better enforcement tools too (Anonymous, 2002). Several international organizations such as Codex Alimentarius Commission (CAC), Food and Agricultural Organization/World Health Organization (FAO/WHO) also keeping their efforts for suitable commitment. International Commission on Microbiological Specification for Foods (ICMSF), International Organization for Standardization (ISO), Office International des Epizootics (OIE) and Commission of European Union (EU) also need to strengthen their commitment on regulatory enforcement in view of public safety issue and global trade. European Union microbiological meat standards are shown in Table 1.

The Codex Alimentarius Commission (CAC) is the reference agency of World Trade Organization for disputes involving food. This committee achieved a great deal of international consensus on food export or import inspection and certification system. So, if one country is willing to export meat and meat products to other country, they need regular monitoring of food products according to international standards or guidelines set by that country, since it is scientifically impossible for importing country to inspect or test the safety of all foodstuffs.

However, the importing country can maintain a limited inspection and sampling program for vigilance against accidental or intentional contamination (JECFA, 2002).

In India, regulations of microbial contaminants fall under the aegis of some Government/Non-Government Organization that is responsible for formulation of standards and monitoring their quality. These are, Prevention of Food Adulteration Rules, 1954, Amendment, 2004; Raw meat (Chilled and frozen) Grading and Marketing Rules, 1991; Bureau of Indian Standard, 1995; Agricultural and Processed Food Export Development Authority (Govt. of India) and Meat Food Products Order, 1973, Amendment, 1994. Microbiological standards under PFA rules, 1954, Ammended 2004 are shown in Table 2.

Table 1: EU microbiological meat quality standards

Meat type	n	c	m	M
Carcass of cattle				
Aerobic mesophilic counts	5-10	-	3.5 log cfu g ⁻¹	5.0 cfu g ⁻¹
Enterobacteriaceae	5-10	-	1.5 log cfu g ⁻¹	2.5 cfu g ⁻¹
Minced meat				
<i>Salmonella</i> sp.	5	0	-	-
<i>E. coli</i>	5	2	50 g ⁻¹	500 g ⁻¹
<i>S. aureus</i>	5	2	100 g ⁻¹	1000 g ⁻¹
Aerobic mesophilic bacteria	5	2	500000 g ⁻¹	5000000 g ⁻¹

Source: Todd (2003)

Table 2: Microbiological standard under PFA rules, 2004

Bacteria	Permissible limits
Total plate counts	100000 g ⁻¹
<i>Escherichia coli</i>	100 g ⁻¹
<i>S. aureus</i>	100 g ⁻¹
<i>Salmonella</i> sp.	Absent in 25 g meat
<i>Listeria monocytogenes</i>	Absent in 10 g meat

Source: MOHFW (2004)

CONCLUSION

In the process of converting live animals into meat, microbial contamination of carcass surfaces is unavoidable. While, most of the microfloras transferred to the carcasses during the slaughtering process are nonpathogenic, there is possibility that pathogens such as *Salmonella* sp., *Escherichia coli* O157:H7, *Campylobacter* sp. and *L. monocytogenes* may be present and it represents one of the most critical safety challenges for the meat industry. Moreover, in recent years with the increase in global trade and awareness of the consumers of the hygienic quality of meat, international attention is being focused on ways to improve the microbial quality and safety of foods. However, to evaluate the effectiveness of any intervention strategies, it is necessary to know the microbial status of the product before and after implementation of the intervention. The present review confirmed the importance of maintaining good process hygiene at meat packing plants for further improvement of microbiological status of meat.

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