DISCUSSION

Microbial contamination reduces the shelf-life of foods and increases the risk of food borne illness. Traditional methods of preserving foods from the effect of microbial growth include thermal processing, drying, freezing, refrigeration, irradiation, modified atmosphere packaging and adding antimicrobial agents or salts. Unfortunately some of these techniques cannot be applied to some food products, such as fresh meats and ready-to-eat products. Outbreaks of food-borne diseases have led to considerable illness and even death (Albrecht, 1986; Lecos, 1987). The living animal carries pathogenic bacteria, while the processing environment harbors them. Bacteria originating from the animal during slaughter, contaminate the carcass and subsequently are distributed via raw meat during further processing (Borch & Arinder, 2002). One efficient way to decrease the microbial contamination on the surface of the meat is effective washing (Crouse et al. 1988; Kotula et al. 1974). Although methods and devices have been developed to clean animal carcasses (Anderson et al. 1982a; Anderson et al. 1982b; Anderson et al. 1983), complete sterilization has not been achieved.

Microbial contamination in commonly marketed animal food products is one of the serious problems faced by developing countries, including India. This study was carried out to survey air and water microflora prevalent in slaughter houses in Bangalore by selecting four major slaughter houses. Subsequently, seven raw meat samples and seven finished meat products were analysed for bacterial contamination. Predominant isolates from all the samples were characterized through biochemical analysis and identified by 16S rRNA gene amplification and
BLAST analysis. Results showed the presence of five common food pathogens at all the four slaughter houses and as well as in raw meat and finished meat products. Further, attempt was made to develop safe measures for preserving meat samples over longer duration, to avoid bacterial contamination.

**Bacterial population by air exposure method**

Slaughter houses are initial source of contamination of meat and meat products. The Russel market is situated in the center of Bangalore city, thickly populated and consists of dirt, dust with a poor hygiene conditions. The workers managing the slaughter process are not trained to handle meat and meat products hygienically. The animal procurement area of these markets is also not clean. Tannery market is located towards north of Bangalore city, densely populated and have no proper hygienic conditions. The meat outlets are also not kept hygienically. This poses a great hazard to public health. The K.R market is in central Bangalore. The market area is unclean for the sale of meat products. The facilities available at meat markets are not good enough to keep the meat fresh for longer time. The Johnson market is located in east of Bangalore city. The market waste disposal management is poorly maintained. The wet waste is thrown in and around of the market area.

Microorganisms are the primary sources of indoor air contamination. The indoor air environment potentially poses a greater risk for contamination than the outside environment, because enclosed spaces can confine aerosols and allow them to build up to infectious levels. Indoor biological pollution has recently received greater scientific
attention. The apparent lack of interest in the studies are linked to the difficulties in sampling biological aerosols and the evaluation of their variable health as well.

Meat is processed in abattoir which invariable differs from place to place but they are principally a place where livestock are slaughtered (Marriott, 2004). A number of slaughter facilities are found in an abattoir, whether stationary or mobile. These facilities could be a source of contamination for the slaughtering processes. It has been reported that abattoir is not 100% hygienic (Gill & Jones, 2005). Different factors are shown to contribute for the contamination of beef products processed in abattoirs, especially during processing. In most developing countries, the traditional methods of handling, processing and marketing of meat undermine the quality, whereas poor sanitation leads to considerable loss of product as well as increase the risk of food-borne disease (Garcia, 2007). Bacteria which are responsible for food borne diseases contaminate meat directly or indirectly, especially from animal excreta during slaughter process (Emswillar et al., 1976). The contaminated water, unclean carcasses, equipments, contaminated surfaces and the air have also been reported to pose serious threat to consumers of beef and beef products, especially in developing countries. Waste water from such abattoirs are usually disposed indiscriminately on terrestrial and aquatic environment, thereby posing a serious health risk to the general public. Most importantly, it has also been observed that most of the operators and patrons of abattoirs do not have any knowledge of sanitary practices which further makes the consumers more vulnerable to microbial
infections. Therefore, it becomes imperative to study all slaughter house samples to analyse the prevalence of microorganisms.

The bioaerosol transport bacteria contribute maximally for the contamination of meat and meat products (Kang & Frank, 1974). This however, emphasizes the importance of controlling the airborne contaminations. The determination of levels and types of airborne bacterial contaminants in slaughter houses also offers an alternative and effective method for the control of carcass contamination in slaughter houses. Cross contamination of meat from the carcass and from the environment are most effectively controlled by appropriate changes in the slaughter processes through the implementation of good manufacturing practices (GMP).

A strong co-relation between the carcass and air contamination was observed in the present study. The skin and internal organs of slaughtered animals have been shown to be the important sources of air borne bacteria in slaughter houses as has been reported earlier (Fournaud et. al. 1978; Nottingham et. al. 1974). Air exposure at Russel market for bacterial population showed an increase of 57.15% in degutting area than the procurement area. While, Tannery market showed an increase of 29.99% in degutting area than the procurement area. The K.R market showed an increase of 22.78% in degutting area than the procurement area. The Johnson market showed an increase of 30.38% in degutting area than the procurement area. Overall analysis showed that the air in the degutting area at Russel market was heavily contaminated than other markets. However, all the markets showed presence of microorganism in air.
Borch & Arinder, 2002; Abdalla et al. 2009; Farnsen et al. 1996
found common food pathogens associated under study are: *Salmonella*,
*Clostridium perfringens*, *Staphylococcus aureus*, *Listeria monocytogenes*,
*Serratia marsacenes*, *Clostridium botulinum*, *B. cereus*, *Escherichia coli*,
*Achromobacter spanius*, and *Streptococcus mutans*. From the analysis it
is been suggested that the degutting area is more contaminated with the
similar kind of bacteria than the procurement area, which may be due to
the fact that the degutting area consists of organic waste matter such as
skin, intestine etc. that can act as medium for the growth of bacterial
pathogen (Fransen et al. 1996). Whereas the procurement area is free of
all these organic wastes that lead to the contamination.

**Water sample analysis**

Water plays a key role in the functioning of the ecosystem.
Processing of meat includes a range of activities, from slaughtering to
complex value-addition activities. Like many other food processing
activities, hygiene and quality requirements in meat processing results in
high water usage and consequently high waste water generation.
However, the water used in four slaughter houses of Bangalore, are not
stored in clean container and during processing of meat samples waste
water and clean water are mixed. Sometimes the same water already used
for cleaning is being used for some other purposes. The water used for
cleaning might contain lots of pathogens and it has been shown that the
water used for cleaning contains lots of fats and proteins, which intern act
as media for growth of many pathogens (Fransen et al.1996).
It is reported that at the time of slaughtering of animals and subsequent meat processing, the water used gets polluted with organic matter of animal origin such as protein and fat and such organic sludge is a sink for multiplication of microorganisms associated with food poisoning. Fransen et al. (1996) have shown a lower microbiological contamination of sludge and raw sludge in poultry slaughter houses. They further showed that the slaughter house sludge was heavily contaminated with *Enterobacteriaceae* and *Enterococci* and also the presence of *Clostridia* and *Salmonella* in the sludge at all the slaughter houses analysed. In the present study, the analysis of water sample at Russel market for bacterial population showed an increase of 30.53% in the water sample after use than the water sample before use. The Tannery market showed an increase of 22.81%, the K.R market showed an increase of 18.61% and the Johnson market showed an increase of 32.00%. Overall, the analysis showed that the water samples after use in Johnson market had higher bacterial population than that of other markets.

The present study corroborates to the fact that food borne illness has been a major concern, especially relating to meat contamination. It is reported in the present study that there is a presence of higher CFU in water samples after use than that of water samples before use. The presence of bacterial pathogens in the water samples shows clearly the presence of water borne pathogenic bacteria in slaughter houses in Bangalore. Among the four major slaughter houses investigated, the much crowded Johnson market slaughter house has been more contaminated with water borne pathogens (CFUs) compared to the other slaughter houses. Hence, from the present study it can be surmised that the storage
of water and heavy human activities are the major causes for contamination of meat and meat products. This confirms the earlier reports of Fransen et al. (1996) who reported that air and water borne bacteria can cause contamination to the slaughter houses. The prime measures for the control of bacterial contamination could be the adoption of HACCP (by analyzing hazard critical points), practicing good personal hygiene, cleaning of the slaughter houses (Swanenburg et al. 2001) and washing truck of trailers (Rajkowski et al. 1998).

**Bacterial population in raw meat samples**

It is reported that meat is one of the most perishable foods. Along with the physical damage, the main spoilage symptoms are the undesirable growth of microorganisms to unacceptable levels. Several groups of microorganisms potentially contribute to meat spoilage under appropriate conditions (Ercolini et al. 2006). Within a certain range of environmental conditions, often only one microbial species from the total microbiota is responsible for the spoilage. Temperature is one of the major factors that influences microbial spoilage influencing the maximum specific growth rate of microorganisms.

Among the raw meat samples analyzed from all the four markets, the sheep feet sample showed the maximum number of bacterial colonies. The sheep feet samples had higher bacterial population which was 78.44% more than raw mutton, 72.55% more than sheep liver, 66.67% more than minced mutton, 64.71% more than sheep lung, 18.63% more than both sheep head and sheep intestine.
Achromobacter spanius is a gram negative rods Achromobacter xylosoxidans is widespread in aquatic habitats, but has also been involved in opportunistic infection of humans (Kersters & De Ley, 1984). But unfortunately there are reports stating isolation this species from food samples. Serratia marcescens, a gram-negative bacillus classified as a member of the Enterobacteriaceae, it is a widely distributed saprophytic bacterium, and has been found in food (Hejazi & Falkiner, 1997). Salmonella enterica is a gram-negative rod-shaped intestinal bacterium belonging to the family Enterobacteriaceae. A slaughterhouse associated outbreak in 1953 when Salmonella contaminated meat caused almost 9000 cases of illness and 90 deaths in humans, clearly showed that Salmonella is a serious disease. B. cereus is a Gram-positive, motile, facultative, aerobic sporeformer. The B. cereus was isolated from many food products like, milk, seafood, rice and some ready to eat food samples (Rahmati & Labbe, 2008; Ankolekar et al. 2009; Rosenquist et al. 2005). Streptococcus mutans are gram-positive cocci shaped bacteria. These facultative anaerobes are commonly found in the human oral cavity, and is a significantly contributor of tooth decay (Whiley & Beighton, 2013).

However, all five isolates were found most abundant in finished products. Hence it is observed that Russel market does not follow proper hygiene, this might be the reason for the higher number of bacterial count. However, sheep feet sample revealed more bacterial count than other raw meat samples this could be due to improper handling and processing (Von Holy et al. 1992). Further, a healthy animal may harbor pathogenic bacteria on its hide, hair, and hooves, in its intestinal tract, and around the lymph nodes (Ayres, 1955; Gill & Newton, 1978).
Bacterial population in finished meat samples

Contaminated meat, meat products and water are among the recognized vehicles for spreading the infection to humans. To reduce the impact of toxigenic isolates, their epidemiology must be fully established. Hence, PCR based methods are the most modern and reliable techniques to detects the number of pathogens in food, water, and environmental materials. Domestic animals, especially sheep and cattle are the main reservoirs and sources of *E. coli* infection for human beings. Bacterial food poisoning is the most common type of food poisoning and it is caused as a result of the presence of harmful bacteria or poisonous substances produced by them in the food. An outbreak of food poisoning may be caused by microorganism which appears to be quite different from those involved in food spoilage. Harmful bacteria (pathogens) find their way into food in number of ways, which is supported by the present findings that air and water are the channels for the entry of meat spoiling bacteria into the meat and meat products. This is also shown through the present studies that the water samples before use was already contaminated with many bacterial population, which might have occurred due to unhygienic conditions. The finished meat samples analysed in the present study at all the four markets. Shared maximum number of bacterial colonies in mutton kofta sample. The percentage difference of mutton kofta with that of other finished products was found to be 62.11% more than mutton samosa, 34.74% more than mutton cubes sample, 27.37% more than mutton burger sample, 24.22% more than mutton sheek
sample, 27.37% more than mutton dried kabab sample, and 22.11% more than mutton biryani sample.

High risk foods are that which are vulnerable to infection by pathogens and as well as foods intended to be eaten without cooking, such as meat, fish, eggs, poultry, milk etc., may be due to the toxins produced by the bacteria. However, in the present study no consistency was observed in the bacterial count in finished meat products in the four markets surveyed. It is interesting to note that bacterial populations were observed even in the finished products. This is in conformity with the earlier findings of Cerveny et al. (2009). It is also observed that the bioload was more in finished product (Mutton Kofta) than the raw meat samples. This could be because of bacterial contamination from the air, as the samples were kept outside in open area. Unhygienic environment and improper handling of finished meat products might have lead to the higher contamination.

It has been reported that the major types of pathogenic bacteria associated with foods are: *Salmonella*, *Clostridium perfringens*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium botulinum*, *B. cereus*, and *Escherichia coli* (Lin et al. 2004; Nychas & Tassou, 1997). Individuals who have recently suffered an attack of food poisoning and still harbouring the organisms in their body can act as source to pass on to other individuals.
Identification of isolated bacteria

There are many methods for identifying bacteria. Traditionally a morphological or biochemical approach has been used. Bacteria are categorized as "Gram Positive" or "Gram Negative" according to whether or not they are stained by a chemical dye, a common biochemical technique. Currently, molecular methods for identification are often used in addition to or instead of biochemical techniques.

Culture-based methods are useful to identify possible pathogens and prediction of the associated food pathogens. However, advances in molecular biology in the last decade have accounted for nucleic acid typing techniques to study of microbial communities. In particular, polymerase chain reaction (PCR) amplification of the bacterial 16S ribosomal RNA (rRNA) gene, followed by cloning and sequencing of the inserts, have resulted in obtaining advanced informations on bacterial diversity.

The 16S rRNA molecule is a basic constituent of the bacterial ribosome, which is a highly conserved structure. The rRNA molecules contain several functionally different regions, some of which have highly conserved sequences, and others have regions of highly variable sequence. By designing PCR primers complementary to conserved regions of the rRNA gene, the approach may be useful in identifying fastidious, uncultivable and novel microorganisms. After cloning and sequencing of the PCR products, the 16S rRNA sequences are compared with other known 16S rRNA sequences to establish phylogenetic affinities and place the organism of interest within a phylogenetic tree. The purpose
of the present study was to examine the presence of bacterial species in meat samples by using PCR technique targeting the 16S rRNA gene, followed by cloning and sequencing (da Silva et al. 2006). Molecular method used in this study like 16S rRNA analysis of the isolates showed it is possible to identify and characterize the bacterial pathogens. This was evident with analysis of all the samples collected from different market places, which showed the presence of bacterial population. This suggests that new molecular technologies are needed for surveillance of food-borne disease and food monitoring.

Furthermore, earlier studies showed that *Serratia sp.* and *Salmonella sp.* were predominant pathogens in meat products (Lin et al. 2004) and was corroborated through the present study, which also showed the presence of these two species. Meanwhile, *Achromobacter sp.* was also among the predominant pathogens found in the all the meat samples, which is less frequently found in contaminated food samples. Further, present analysis showed that all these pathogens frequently occur as contaminants in fresh and finished meat products.

In the present study, out of the 2 air samples, 2 water samples, 7 raw meat samples and 7 finished meat product samples, a total of 540 bacterial colonies were isolated, and preliminarily identified based on colony characteristics and biochemical tests. However, 5 predominant colonies were conspicuous by their presence in all the samples at all the four markets. These 5 colonies were selected for further analysis through 16S rRNA gene sequencing and were designated as SAA1, SAA2, SAA3, SAA4 and SSA5 respectively. After 16S rRNA gene sequencing and construction of phylogenetic tree, the 5 predominant colonies were
identified as *Achromobacter spanius* SAA1, *Serratia marcescens* SAA2, *Salmonella enterica* SAA3, *Bacillus cereus* SAA4, and *Streptococcus mutans* SAA5.

**Safety measures**

Although special care is taken at the processing plant, there are always some bacteria sticking on to the meat surface which cause spoilage and sometimes food borne illness. Many techniques are available so far to help preserve meat attributes. They may be used alone or in combination with others. Methods used to reduce meat spoilage caused by bacteria that are unavoidably deposited on its surface can be divided into two categories: (a) Reduction or inhibition of growth through temperature control. (b) inactivation of microorganisms, through use of chemicals.

**Exposure to cold temperature**

Reduction of temperature below 0°C, causes the water content of the product to be converted to solid state. Also, the effect of temperature is not the same on all types of microorganisms. In fact, the rate at which bacterial growth decreases with decreasing temperature varies according to species and strains of microorganisms (Lambert et al. 1990). The ideal temperature for the growth of microorganisms is between 7 °C and 55 °C. At temperatures above 80°C, the microorganisms are usually killed. Spores are often resistant to temperatures above 100 °C. Animal products generally remain fresh in the refrigerator (2-4°C) for 4-7 days and they can be stored much longer in the deep-freeze at -20°C (Berkel et al. 2004).
The present results on the effect of cold temperature surmised that raw meat samples stored at -5 °C showed no bacterial population. Therefore, it could be suggested that low temperature below -5 °C would be ideal for storage of raw meat samples. However, this method requires a lot of energy and materials and a large investment (Berkel et al. 2004).

**Pickling of raw meat**

The use of salt to preserve foods is a traditionally developed practice dating back to thousands of years. Although primarily evolved to prevent spoilage, salting also prevents the growth of food poisoning organisms. Salting is very important as it enhanced the nutritional benefits of meat, fish and vegetables.

The development of other preservation techniques, such as refrigeration, envisage that salting is no longer necessary and foods using salt as the primary preservation mechanism have become relatively uncommon, as they are shown to make the preserved food too salty. However, uses of salt for partial preservation are still in practice, because of their organoleptic properties and their functional properties in food. Preservation is based on slowing down or preventing spoilage by microorganisms (Stringer & Pin, 2005). Thus contaminations by microorganisms can be avoided. Therefore, the present study was carried out for meat preservation with different salt concentrations ranging from 2 to 8%. Samples pickled at 6% of salt had no bacterial population. Therefore, it is suggested that pickling of meat samples with salt at 6% or above would prevent meat samples from spoiling.
Combination of cold storage and pickling

In order to analyze the combined effect of low temperature and salting, the experiment was designed by adopting both low temperature and pickling of meat. Growth responses as a function of the combination of NaCl and low temperature were described to predict microbial growth.

Therefore, from the results it could be suggested that storage of meat samples at 4°C and 8% salt combination could avoid the bacterial contamination. The present approach carried out in this study was to store the meat effectively to extend its shelf life as was previously attempted by Stringer & Pin (2005).

Based on the present study it can be concluded that the microbial quality of the raw meat could be determined through the maintenance of cold chain, sanitary condition of premises, equipment and personnel and general management practices. Presence of all the five identified food pathogenic species in finished product emphasizes the lack of hygienic conditions being maintained at the food outlets.