Chapter VI.

Summary and Conclusions

Bacterial foodborne pathogens are a worldwide cause of morbidity and mortality. Human illness from milkborne pathogens is usually associated with consumption of raw milk or products made from raw milk. Milk contains many
essential nutrients, such as carbohydrates, proteins, lipids, minerals and vitamins and therefore acts as an ideal medium for rapid proliferation of harmful organisms (pathogens as well as spoilage organisms). Milk may become contaminated with bacteria during or after milking. Production of milk and its products involves a long sequence of operations from harvesting to final consumption during which it is exposed to various microorganisms. Microbiological examination of milk is essential to find the degree of contamination. The assessment of microbial load at various stages of manufacture or processing may serve as a useful tool for quality assessment and improvement which will result in longer shelf life which is a desirable market requirement. Laboratory based surveillance performed in a timely manner, exploiting adequate methods and cooperation at local and national levels are among the key elements in preventing food-borne diseases. The availability of subtyping procedures to track individual strains involved in outbreaks, and to examine the epidemiology and population genetics of bacteria, is integral to control and prevention programs aimed at limiting infections. Application of subtyping methods also provides insight into the population genetics, epidemiology, ecology, and evolution of bacteria.

In the present study, a total of 933 samples comprising of milk from dairy animals collected at different levels of collection and processing (udder, milking utensils, dairy cooperative society (DCS), receiving dock and bulk coolers) and swabs from cans and milk processing line as well as environmental samples within Goa region during different seasons were analyzed for microbiological parameters. The samples were analysed for standard plate count (SPC), coliform count,
methylene blue reduction tests and California mastitis test. The samples were further analysed for isolation of *Escherichia coli* and *Listeria*. The presumptive isolates of *E. coli* and *Listeria* were further confirmed by biochemical tests and serotyping. Molecular characterization of *E. coli* and *Listeria* was also carried out. Antibiotic susceptibility testing of *E. coli* and *Listeria monocytogenes* isolates was performed. Swab samples of milk cans and processing unit were also analyzed for SPC and coliform count. Highest counts were detected from samples collected at receiving dock and with regard to season, the highest counts were obtained during the period from March to May. The MBRT time decreased as the time elapsed from milking to processing. CMT analysis showed a varied response.

The bacteriological examination of milk samples (n=767) revealed *E. coli* 77 (10.04%) samples. Out of 77 *E. coli* isolates, 64 were typeable and belonged to 35 different O serogroups of which O4 was the predominant. Of 77 *E. coli* strains recovered, 25(32.47%) had one or more of the genes responsible for virulence of *E. coli* (Table 4.2). PCR assay revealed that 11 of 77 (14.29%) of the isolates carried the stx1 gene alone, 3 of 77 (3.9%) possessed the stx2 alone and 11 of 77 (14.29%) carried both the stx1 and stx2 genes. A multiplex PCR to detect the presence of 7 virulence genes in *E.coli* namely P-fimbriae (*papC*), iron-repressible protein (*irp2*), temperature-sensitive hemagglutinin (*tsh*), vacuolating auto transporter toxin (*vat*), entero-aggregative toxin (*astA*),increased serum survival protein (*iss*), and colicin V plasmid operon genes (*cvi*) revealed a varied pattern of the presence of these genes in *E. PFGE of Xbal-digested DNA fragments isolated from the 74 *E coli* isolates generated 54 unrelated *XbaI-PFGE subtypes at 60% similarity* *coli isolates.*
The isolates showed varying degree of susceptibility to the antibiotics. In conclusion, our findings provide the information about the involvement of STEC in raw milk in India. In India, a large number of people in rural areas and a much larger segment of the population consume raw unpasteurized milk directly and indirectly. The fact that the serotypes reported from raw milk samples were common to that reported from animal populations suggests that the source of contamination of milk might be from animals.

Overall, 10.56% of the samples (81 of 767) were positive for *Listeria* species. Out of these 37 (4.82%) were confirmed as *L. monocytogenes*. Other *Listeria* species isolated were *L. innocua* (5.47%), *L. ivanovii* (0.13%) and *L. grayi* (0.13%). Maximum isolates were recovered from samples collected from market followed by samples at milk processing unit. *L. monocytogenes* were serotyped using multiplex PCR method. A larger proportion of isolates (26) belonged to group corresponding to serovars 1/2a, 1/2c, 3a, and 3c. Serogroup corresponding to serovars 4b, 4d and 4e were detected in two strains while serogroup 1/2b, 3b, 4b, 4d, and 4e were detected in nine strains. PFGE discriminated the *L. monocytogenes* isolates into 5 *Apa*I and 4 *Ascl* PFGE patterns (pulsotypes) at 80% similarity, but could differentiate serovars within MPCR serogroups, in which isolates from different serovars displaying the same pulsotype were found. PFGE analysis of isolates from different locations in India showed that the profiles constitute a homogeneous population. Out of 37 *L. monocytogenes* isolates, 16 were found resistant to penicillin, 8 to vancomycin and 6 to ampicillin. The occurrence of pathogenic *L. monocytogenes* in raw bovine milk is of great concern from public
health point of view as they can serve as a source for the transmission of human listeriosis. This occurrence of pathogenic *L. monocytogenes* in milk could be as a result of unhygienic conditions and improper husbandry practices. This calls for an urgent need of hygienic measures to be adopted at farm level as well as at dairies.

This study shows the prevalence of pathogens of public health significance in raw and market milk and the need for good hygienic practices to prevent their entry into the food chain. In conclusion, effective food safety interventions to reduce or control foodborne pathogens are needed throughout the food continuum, from the farm to the end user. Current production and processing procedures for livestock and their products do not have sufficiently robust food safety interventions to ensure pathogen-free raw milk and products.

**Need for Future Research**

Bacterial foodborne pathogens are a major cause of morbidity and mortality throughout the world. The broad spectrum of food-borne infections has changed
dramatically over time, as well-established pathogens have been controlled or eliminated, and new ones have emerged. The successes of the 20th century and the new challenges we face mean that public health vigilance, careful investigation of new problems, responsible attention to food safety from farm to table, and partnerships to bring about new food-borne disease control measures will be needed for the foreseeable future (Tauxe, 2002).

Quality management on dairy farms becomes more and more important regarding the different areas of animal health, animal welfare and food safety. Monitoring animals, farm conditions and farm records can be extended with risk identification and risk management. The hazard analysis critical control point's system is useful as an on farm strategy to control the product as well as the production process on the areas of animal health, animal welfare and food safety.

Interventions that are applied shortly before milking and during processing have the potential to reduce contamination of milk, but measures that reduce carriage and shedding of STEC by ruminants have the potential to also affect infections because of secondary contamination of foods, drinking water, or to direct contact with animals. Studies on types of STEC and pathogenesis of the organism contribute to our understanding of the disease process and our ability to diagnose and treat infections. However, major emphasis in research and application is on prevention of human infection with STEC, and many steps have been taken worldwide to reduce human infection with STEC, especially serotype O157:H7. These measures involve introduction of procedures at the postharvest level and educating the public.
Research has revealed that enteric pathogens can survive as endophytes or epiphytes, but this relationship differs among bacterial species as well as plant cultivars. Research on the behavior of many genotypes of enteric pathogens as well as their interaction with numerous plant cultivars is just beginning. These findings may help identify critical factors affecting colonization of produce by foodborne pathogens. Future research should focus on elucidating the ecological factors that contribute to epiphytic and endophytic relationships of foodborne pathogens. This is important as green fodder may be involved in transmission of enteric pathogens to animals.

Farm animals can be asymptomatic or suffer from encephalitis, septicaemia and abortions and thus may be a source of *L. monocytogenes* in the farm environment. To manage the problem of foodborne listeriosis requires an understanding of the burden of the disease on a worldwide scale as foods that are prone to contamination are eaten widely domestically and many are traded globally. Surveillance of the disease, caused by *L. monocytogenes*, is typically restricted to developed countries, but many of these do not consider listeriosis as a notifiable disease and estimate the numbers by other means. The cause of listeriosis is almost always through a food source contaminated somewhere in the food chain (Mead et al., 1999; Norton and Braden, 2007). A major source is raw material coming into a processing facility carrying with it both transient and persistent strains. Some strains have been shown to persist for months or even years (Kathariou, 2002). Ready-to-eat foods are the vehicle for transmission of the *Listeria* through contamination somewhere in the food chain. Meat, poultry and dairy products have
been most frequently implicated, but other foods including produce may also have been vehicles of transmission. In addition to outbreak investigation, case-control studies, and the use of experts, risk assessments, and food attribution studies can help focus on areas of greatest risk for prevention and control measures throughout the food chain.

*Listeria monocytogenes* and resident microorganisms from food industry premises have been found to interact in biofilms. The importance of listeriosis in developing countries has also not been determined. At present, it seems to be a lower priority compared with other public health problems but its surveillance in many parts of the world is very limited. As at least a proportion of the population in the developing countries adopts western customs of purchasing RTE foods and storing them in refrigerators (especially if there are periodic power shortages), *L. monocytogenes* may become a more important pathogen.

Because of their significant impact on human health and well-being, the ability to carry out epidemiological investigations to determine the primary sources of bacterial contamination is important to improve public health. Multiple methods can be used for bacterial source tracking and to determine the distribution of pathogens isolated from ill people. Equally important is the ability to rule out non-related isolates from a particular outbreak, which would likely confound the epidemiological investigation of a foodborne illness outbreak. Bacterial isolates can be characterized based on their phenotypic traits, such as antibiotic susceptibility profiles and serotyping, or by genotypic methods using molecular typing techniques. Electronic database libraries of the different genomic profiles will
enable continuous surveillance of infections and detection of possible infection clusters at an early stage. Laboratory-based surveillance performed in a timely manner and exploiting adequate methods, and co-operation at local, national and international levels are among the key elements in preventing food-borne diseases.

Studies on microbiological surveillance of milk and milk products at farm level may help in management and stamping of herds as clean. Human illness attribution has been recently recognized as an important tool to better inform food safety decisions. All dairy farmers, suppliers to dairy farmers, milk carriers, dairy product and food manufacturers, distributors and retailers should be part of an integrated food safety and quality assurance management system. Good farming practices underpin the marketing of safe, quality-assured milk-based products. Good dairy farming practices should contribute to ensuring milk and milk products are safe and suitable for their intended use.