Summary
Food borne diseases are widespread and growing public health problem in developed and developing countries both. In developing countries including India, the magnitude of food borne illness is very high. The majority outbreaks of food borne disease are unreported, unrecognized or un-investigated. A variety of micro-organisms are associated with food borne diseases *Salmonella*, *Campylobacter jejuni*, *S. aureus*, *C. perfringens* and *Y. enterocolitica* *Bacillus cereus*, *E. coli*, *Enterobacter spp.* faecal *coliforms*, *Listeria spp.*, *Shigella spp.*, *S. aureus*, *S. Typhi*, *Streptococci spp.*, *Vibrio spp.* and *Yersinia spp.* (Bansal and Kaul, 2004; Amruthasri and Devi, 2005; CD Alert, 2009). Food safety has been recognized as major issue with international trade and public health implications both at national and International level (Sudershan et al., 2009). There is serious concern required about the communication of food borne diseases and early identification of the pathogen with the aim of preventing these hazards from becoming real risks and causing diseases (Kleter and Marvin, 2009; ICEID, 2012).

Conventional bacterial identification methods rely on selective media and regarded as golden standards for the identification of bacterial pathogens. However, these cultural methods are time consuming and labor intensive. Therefore, there are great demands of rapid methods that can be useful to quickly screen large number of samples. The advent of molecular biology techniques has greatly improved food testing method. The polymerase chain reaction (PCR) based methods developed for the detection and identification of food borne bacteria is mainly based on 16S rRNA and virulence gene of specific group of bacteria which provide specific level identification (Linton et al., 1997; Asma et al., 2009; Pourmand et al., 2009; Moussa et al., 2010; Navidinia et al., 2012). The various virulence factors such as hippuricase, listeriolysin, invasion, enterotoxin and verotoxin have been recognized and used specifically for the detection of *C. jejuni*, *L. monocytogenes*, *Salmonella spp.*, *S. aureus* and VTEC respectively. The detection based on virulent genes provides an advantage to direct information about the pathogenic potential of the organism. Both conventional and PCR based methods have their own advantage and limitations however, the development of standardized PCR techniques will be helpful to assess the food borne contamination more quickly and accurately. This requires a systematic assessment of local food samples, both by conventional and PCR based methods to obtain data on prevalence of pathogens in a particular region and to suggest remedies.
The systematic investigation of various foods in majority of northern India here under studied has not been reported previously. Therefore, the present study has planned with following aims and objectives; (i) to study prevalence of common food borne bacterial pathogens in various foods (ii) to assess the bacterial load in selected food samples (iii) to detect *Campylobacter jejuni*, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus* and Verotoxic *E. coli* by standardized PCR methods (iv) to standardize multiplex PCR for simultaneous detection of *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus* (v) to detect *Campylobacter jejuni*, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus* and Verotoxic *E. coli* in spiked food samples by cultural and PCR methods (vi) to assess the antibiotic resistance profile among selected strains of food borne bacterial pathogens. The major findings of the present investigation are summarized below;

**Isolation and biochemical characterization of food borne bacterial pathogens from collected samples**

In the present study, a total of 650 samples from various food sources were subjected to isolate five food borne bacterial pathogens (*Campylobacter jejuni*, *L. monocytogenes*, *Salmonella* spp., *S. aureus* and *E. coli*) using standard procedure of USFDA. A total of 79 pathogenic *E. coli* isolates were confirmed for cytotoxicity assay on Vero cell lines. Of these 79 *E. coli* isolates, only 20 isolates showed a cytopathic effect on Vero cells and characterized as Verotoxic *E. coli* (VTEC). Among pathogenic isolates the serotype O60 was found to be most frequent serotype isolated in this study.

**Prevalence of bacterial pathogens in food samples**

Based on cultural and biochemical methods the overall prevalence of *C. jejuni* was found 13.09% (72 isolates) from total collected 550 samples. Similarly, occurrence of *L. monocytogenes* (3.8%), *Salmonella* spp. (7.4%), *S. aureus* (18.5%) and pathogenic *E. coli* (26.3%) were isolated from their total respective samples screened for individual bacterial pathogen. The prevalence of *C. jejuni* was observed highest (38.6%) from raw chicken meat followed by beef (10%), fish meat (4%) and raw milk (0.6%). Similarly, *L. monocytogenes* was recovered highest (6.0%) from raw chicken meat followed by fish meat (4%), beef (2.5%), curd (2%) and raw milk
Salmonella spp. were found highest (11%) in fish meat followed by raw chicken meat (8%), beef (4%) and raw milk (3.3%). The highest (36%) occurrence of S. aureus was confirmed in beef which was followed by raw chicken (21.3%), fish meat (15%) and raw milk (14.6%). In our study, E. coli was recovered highest from beef (44%), raw chicken meat (38%), fish meat (34%) and raw milk (14%).

**Enumeration of bacteria in food samples**

Food samples found positive for the presence of one or more groups of pathogenic bacteria were selected to assess the microbiological quality of raw meat, raw milk and milk products in terms of viable counts on the following parameters;

The total aerobic plate count for various food samples understudied was from log 3.0 to log 7.8 cfu g⁻¹. In this study, 73.5% samples showed count between log 6.0 to log 6.9 cfu g⁻¹ among beef samples whereas majority of fish samples 60.2% showed count between log 5.0 to log 5.9 cfu g⁻¹. Whereas in raw chicken meat samples where 35.4% samples showed TAPC of ≥log 6.0 cfu g⁻¹.

The total coliforms observed from various food samples varied between log 1.0 to log 4.8 cfu g⁻¹. In our findings, 76.4% beef samples, exhibited values from log 3.0 to log 3.9 cfu g⁻¹, whereas, among fish meat samples majority of samples (63.6%) showed count between log 3.0 to log 3.9 cfu g⁻¹. A large amount (82.7%) of samples showed count of ≤ log 3.0 to log 3.9 cfu g⁻¹ in raw chicken meat. In all (100%) raw milk and curd samples the coliform count was found between log 2.0 to log 2.9 cfu g⁻¹.

In the present study, Campylobacter load of log 1.0 to log 5.8 cfu g⁻¹ was evaluated. Among beef and fish meat (69.2% each) showed count between log 2.0 to log 2.9 cfu g⁻¹. Overall, 65.5% raw chicken samples showed count between log 2.0 to log 3.9 cfu g⁻¹.

The enumeration of L. monocytogenes, the count in various food samples was found between log 1.0 to log 3.8 cfu g⁻¹. Among beef samples the L. monocytogenes count was varied from log 1.0 to log 2.9 cfu g⁻¹ whereas among fish meat samples varied from log 1.5 to log 2.8 cfu g⁻¹. The load of L. monocytogenes was observed between log 1.7 to log 3.8 cfu g⁻¹ among raw chicken meat samples, whereas, two raw milk samples screened showed L. monocytogenes count of log 3.8 cfu g⁻¹ and log 4.0 cfu g⁻¹.
The presence of *S. aureus* among various food samples was evaluated between log 1.0 to log 4.8 cfu g⁻¹. The pathogen was traced between log 3.0 to log 3.8 cfu g⁻¹ among maximum (68.1%) samples.

The *E. coli* load was found between log 1.5 to log 3.9 cfu g⁻¹ for various food samples. Overall, 84.2% samples showed count between log 2.0 to log 3.8 cfu g⁻¹. The enumeration of *E. coli* in raw milk found that 90.4% samples showed the *E. coli* count between log 3.0 to 3.9 cfu g⁻¹.

**Detection of bacterial pathogens based on specific gene by PCR isolated from food samples**

A total 319 bacterial isolates were obtained by cultural method. Further, the presence of specific gene confirmed by PCR for *Campylobacter jejuni* (hip’O’), *L. monocytogenes* (hlyA), *Salmonella* spp. (invA), *S. aureus* (seA) and Verotoxic *E. coli* (vt₁ & vt₂). The standardized hip’O’ gene specific reaction for *C. jejuni* generated a clear band at 735 bp. The *L. monocytogenes* isolates identified on the basis of hlyA gene specific PCR assay, which generated a clear band at 234 bp. The PCR product of 389 bp was obtained after standardized PCR assay targeting invA gene for *Salmonella* spp. identification. The seA gene based PCR assay for *S. aureus* identification generated a PCR product of 120 bp. Similarly, VTEC were identified targeting vt₁ and vt₂ genes in separate PCR assay. The PCR products of 349 bp and 478 bp were generated from these standardized PCR assay.

Food borne pathogens (*C. jejuni, L. monocytogenes, Salmonella* spp, *S. aureus* and pathogenic *E. coli*) isolated and characterized biochemically were subjected to PCR analysis for the detection of specific gene such as hip’O’, hlyA, invA, seA and vt₁ & vt₂. The PCR assay was turned out positive in 68 (94.4%) *C. jejuni*, 25 (100.0%) for *L. monocytogenes*, 41 (100.0%) for *Salmonella* and 29 (28.4%) for *S. aureus* and 20 (25.3%) for VTEC.

Overall, the detection of hip’O’gene was confirmed in 68 (12.3%) isolates for *C. jejuni* from total samples collected. The presence of hlyA gene was confirmed in 25 (3.8%) *L. monocytogenes*. Similarly, in 41 (7.4%) *Salmonella* isolates, invA gene assay was turned out positive. The presence of seA gene was confirmed in 29 (5.2%) *S. aureus* whereas 20 (6.6%) for VTEC.
**Multiplex PCR (m-PCR)**

In this study, a multiplex PCR assay was designed for the simultaneous detection of *Salmonella*, *S. aureus* and *L. monocytogenes* in beef, cheese, raw milk samples. The samples were spiked and screened for detection of 3 bacterial pathogens (*Listeria monocytogenes*, 163 bp of origin of replication (oriC) of *Salmonella Typhimurium* and 270 bp of nuclease (nuc) gene of *Staphylococcus aureus* simultaneously both by cultural and multiplex PCR method. The concentration of 2.5 mM MgCl$_2$, 0.4 mM dNTPs, and 10 pmol of each primer were used to give optimal results.

The sensitivity of standardized m-PCR assay was evaluated by heat lysed DNA from 10 fold serially diluted standard cultures of *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus* from beef, cheese and raw milk. The minimum level of detection was found to be 10 cells/ml in all food samples examined.

**Cultural and PCR methods in spiked food samples**

The cultural and PCR methods based detection was carried out in spiked food samples. Analysis revealed that all bacteria were detected upto 10 cells/ml in all food samples by cultural and PCR method both except in case of *C. jejuni*, where the detection upto 100 cells/ml was found positive by cultural methods. However, PCR was turned out positive upto 10 cells/ml in both types of food samples.

**Antibiotic resistance of isolates**

A total of 119 randomly selected isolates of *C. jejuni*, *L. monocytogenes*, *Salmonella spp.*, *S. aureus* and Verotoxic *E. coli* from total PCR confirmed isolates (183) were examined for their antibiotic resistance pattern. Therefore, different pathogens were tested against a set of antibiotics The *C. jejuni* isolates (30) from various food sources were examined against 9 antibiotics. Overall, the resistance against cephalothin (86.6%) was highest followed by co-trimoxazole (73.3%), chloramphenicol and tetracycline (60.0% each), erythromycin (13.3%), ciprofloxacin and gentamycin (3.3% each). No resistance was observed against azithromycin and nalidixic acid.
Similarly, antibiotic sensitivity against 8 antibiotics was also observed among L. monocytogenes and revealed highest resistance (30%) to penicillin followed by chloramphenicol and tetracycline (20% against each). Only 10% isolates were found resistant to ampicillin, cephalexin, ciprofloxacin, gentamycin and trimethoprim. Among 30 tested Salmonella isolates 10 (33.3%) isolates were resistant to ampicillin and cephalexin each followed by 6 (20.0%) ciprofloxacin, and 5 (16.6%) to tetracycline. It was also found that no Salmonella isolates were resistant to the chloramphenicol, gentamycin, nalidixic acid and streptomycin.

In most of S. aureus isolates, highest resistance (44.4%) was observed to penicillin followed by streptomycin and ampicillin (27.5% each). Chloramphenicol was observed as most effective (3.4% resistance) antibiotic. Among 20 VTEC tested isolates, highest (80.0%) resistant to ampicillin and nalidixic acid was observed which was followed by streptomycin, tetracycline, cephalexin, gentamycin, co-trimoxazole and norfloxacin. In this study, multiple drug resistance (resistance to ≥3 antibiotics) was observed among 15 (75.0%) VTEC, 19 (63.3%) C. jejuni, 07 (23.3%) Salmonella, 05 (17.2%) S. aureus and 3 (3.3%) L. monocytogenes isolates.

The findings of the present investigation may be concluded as follows:

- The microbial contamination of various food samples are in the order of beef>raw chicken > fish meat > raw milk > curd > cheese. In many cases especially beef and raw milk samples where the level of contamination was found above the standard guideline, indicating poor microbiological quality of the food samples.
- The food borne pathogens under this investigation are more commonly encountered in meat cultural methods were found in various frequencies among beef (Pathogenic E. coli > S. aureus > C. jejuni > Salmonella spp. > L. monocytogenes), fish meat (Pathogenic E. coli > S. aureus > Salmonella spp. > C. jejuni and L. monocytogenes), raw chicken (C. jejuni > Pathogenic E. coli > S. aureus > Salmonella spp. > L. monocytogenes), raw milk (S. aureus > Pathogenic E. coli and Salmonella spp. > L. monocytogenes > C. jejuni).
- The bacterial pathogens were characterized further by standardized PCR method targeting specific gene which provided more specific detection and confirmation of these bacterial isolate.
- The standardized PCR methods were further tested in spiked food samples and found effective for the direct detection of these bacterial pathogens.
• To find out the PCR method more effective and economical the multiplex PCR (m-PCR) was developed with the three bacterial pathogens viz. *L. monocytogenes, Salmonella typhymurium* and *S. aureus*. Such m-PCR could be effectively used in spiked food samples.

• Varying incidence of antibiotic resistance against common antibiotics was recorded among selected pathogens studied. Multiple drug resistant (MDR) is also encountered in pathogenic isolates.

**Recommendations and future work plan:**

Significant bacterial contamination among foods under investigation was found. The most probable reason is expected due to poor hygienic conditions and practices adopted by food handlers. Therefore, it is suggested that implementation of Good Manufacturing practices (GMP) and Good Hygiene Practices (GHP) should be ensured to maintain the good quality of foods. In future, the isolated and characterized bacterial pathogens may be further subjected to 16S rRNA analysis, development of more uniplex and m-PCR assay to be used in natural food samples for detection of these bacterial pathogens alone or simultaneously, the role of other virulence factors may be investigated as suitable marker and molecular linkage between drug resistance gene and associated virulence gene may also be explored.