Diarrhea is an important cause of morbidity and mortality in all regions of the world and among all ages (Boschi-Pinto et al., 2008; WHO, 2008). For children 5 years of age and older, adolescents, and adults mild to moderate diarrhea can lead to absenteeism from school or work and may require treatment by a health care provider. More severe diarrhea can lead to hospitalization; serious sequelae such as Guillain Barre' syndrome and hemolytic uremic syndrome; and in some cases death (Amirlak and Amirlak, 2006; Allos, 2001). Diarrhea is defined as stool weight in excess of 200 grams per day. However, this definition is of little clinical value, since collecting and weighing stools is neither practical nor required except in a clinical research setting. A good working definition is three or more loose or watery stools per day or a definite decrease in consistency and increase in frequency based upon an individual baseline.

Diarrhea reflects increased water content of the stool, whether due to impaired water absorption and/or active water secretion by the bowel. In severe infectious diarrhea, the number of stools may reach 20 or more per day, with defecation occurring every 20 or 30 minutes. In this situation, the total daily volume of stool may exceed two liters, with resultant volume depletion and hypokalemia. Most patients with acute diarrhea have three to seven movements per day with total stool volume less than one liter per day. When diarrhea lasts for 14 days it can be considered persistent; the term chronic generally refers to diarrhea that lasts for at least one month (Dupont, 1997). It is estimated that preschool children have diarrhea 10-20 percent of the time or for about 35-70 days each year (Black et al., 1982). Ten percent of the children with diarrhea may become severely dehydrated and 0.5 percent of them may die (Snyder and Merson, 1982). Repeated diarrheal illness has a detrimental effect on growth, leaving the child frail and prone to other infections. It has been estimated that 25
percent of the growth differential between children in developing and developed
countries can be attributed to diarrhea (Black et al., 1984; Martorell et al., 1975).
Of particular importance among diarrheal illness are longer duration episodes
(Black, 1993). The case fatality rate with persistent diarrhea is much higher than
with acute diarrhea, and longer duration episodes have a greater adverse effect
on growth than shorter duration episodes (Black, 1993). Studies have shown that
about one-third to two-thirds of all diarrhea-associated deaths in children in
developing countries follow episodes of persistent diarrhea (Victora et al., 1993;
Fauveau et al., 1992). Possible factors that influence duration of illness include
age, nutritional status, immunologic status, previous diarrheal morbidity, diet,
drugs, and use of rehydration fluids (Black, 1993). It is also possible that events
that occur in the early phase of diarrhea may set the stage for more protracted
illness. Given that the epidemiology of long duration diarrhea is only partially
understood, the identification of risk factors for longer illness duration even when
episodes do not meet the definition of persistent diarrhea may provide important
clues to its pathogenesis and prevention. The clinical manifestation of infection
depend on factors such as the physiological state of the host and the pathogenic
properties of the particular strain (Cover and Aber, 1989).

Foodborne diseases are widespread and a growing public health problem
in developed and developing countries (Schlundt, 2002). Human yersiniosis is
attributed to contaminated pork, milk, water, and tofu consumption, as well as
blood transfusion. Infected individuals may shed Y. enterocolitica in stools for 90
days after the symptom resolution, suggesting that early detection of Y.
enterocolitica from diarrheal stool samples is critical in preventing its
transmission and an eventual outbreak (Cover and Aber, 1989).

Yersinia enterocolitica is the frequently reported zoonotic gastrointestinal
disease after campylobacteriosis and salmonellosis in many developed
countries, especially in temperate zones. Within developed countries, incidences
of yersiniosis and foodborne outbreaks appear to be lower in the United States
than many European countries (Bottone, 1997; Cover and Aber, 1989). In
European countries, number of reported cases of human in England and Wales
are lower than those in other European countries where fewer than 0.1 cases of yersiniosis per 100,000 individuals were reported in the United Kingdom in 2005, in contrast to 12.2 in Finland and 6.8 in Germany. On the other hand, the high prevalence of gastrointestinal illness including fatal cases due to yersiniosis is also observed in many developing countries like Bangladesh, Iraq, Iran and Nigeria (Butler et al., 1984; Kanan and Abdilla, 2009; Soltan-Dallal and Moezardalan, 2004; Okwori et al., 2009) which indicates major underlying food safety problems in low- and middle-income countries.

The genus *Yersinia* consists of 11 species, three of which can cause disease in human beings and animals. They are *Yersinia enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis* (Bercovier et al., 1984) The former two are transmitted by the faecal-oral route, but later one is transmitted mainly by flea bites causative for plague disease. These three pathogenic species differ considerably in invasiveness. *Y. enterocolitica* and *Y. pseudotuberculosis* can cross the intact gastro-intestinal mucosa and affect the underlying structures. *Y. pestis* infection is vector dependent and it cannot penetrate any body surface on its own (Cornelis et al., 1998).

*Yersinia enterocolitica* is a well-characterized entero invasive bacterial species that causes intestinal and extraintestinal human infections (Prpic et al., 1983). It belongs to family Enterobacteriaceae, a group of gram negative, oxidase negative and facultative anaerobic bacteria. All bacteria belonging to the genus *Yersinia* are catalase-positive, non-spore forming rods or coco-bacilli of 0.5 - 0.8x, 1-3 μm in size (Bercovier et al., 1984).

*Y. enterocolitica* is a facultative intracellular pathogen that causes a broad range of food and water borne gastrointestinal syndromes ranging from mild diarrhea to mesenteric lymphadenitis. Gastrointestinal Yersiniosis occurs most frequently in infants and young children (Bottone et al., 1997), sometimes post infection disorders more specifically extra-intestinal such as reactive arthritis (Granfors et al., 1989), erythema nodosum (Ahvonen, 1970), enteritis, urinary
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and respiratory tract infection, extra mesenteric forms etc are also reported (Aho et al., 1974).

*Y. enterocolitica* is non-lactose-fermenting, glucose-fermenting, and oxidase-negative. Most, but not all *Y. enterocolitica* isolates reduce nitrates. The presence of bile salts in the medium prevents the organism’s ability to ferment lactose. Colonies of *Y. enterocolitica* do not produce hydrogen sulfide in triple sugar iron medium, but the organism is urease-positive. *Y. enterocolitica* is a facultative anaerobe that is motile at 25°C and non motile at 37°C.

Upon initial isolation on enteric media, *Y. enterocolitica* resembles other common Enterobacteriaceae. Using duplicate sets of enteric media followed by incubation at both 25°C and 37°C for 48 hours increases the yield from stool cultures. Cefsulodin-irgasan-novobiocin (CIN) agar is a highly selective medium for this organism. It requires 18-20 hours of incubation at 25°C to create unique colony morphology, representing 0.5 to 1mm colonies with a red "bull's-eye" and a clear border. Use of this media allows differentiation between *Y. enterocolitica* and *Y. enterocolitica* like isolates.

*Y. enterocolitica* is classified according to various distinct biochemical and serologic reactions. Based on biochemical characteristics, 6 biotypes of *Y. enterocolitica* have been described. Biotypes 2, 3, and 4 are most common in humans. The serotyping is based on O and H antigens. More than 60 serotypes of *Y. enterocolitica* have been described. The serotypes most clearly pathogenic to humans include serotypes O:3, O:5,27, O:8, O:9, and O:13. H-antigen typing can be a valuable supplement to O-antigen typing and biochemical characterization in epidemiological investigations. Accurate identification of pathogenic strains requires consideration of both the biotype and the serotype because some strains can contain multiple cross-reacting O antigens. However, infective diarrhea continues to be a leading cause of human pathology and bacteria are responsible for 10–55% of diarrheal episodes with highest rates occurring in the developing world (Mendall et al., 2005).
Y. enterocolitica and Y. enterocolitica-like bacteria constitute a fairly heterogeneous group of bacteria which are ubiquitous in terrestrial and freshwater ecosystems. Pathogenic significance in man is mainly associated with a few serogroups (O:3, O:9, O:8, O:5,27). The pathogenic serogroups show different geographical distributions. The development of isolation procedures which clearly differentiate pathogenic from non-pathogenic variants has been difficult. Of special significance in food hygiene is the ability of Y. enterocolitica to grow in refrigerated foods. There is strong indirect evidence that pigs and food products of porcine origin are the major sources for human infection with Y. enterocolitica serogroups O:3 and O:9, the dominant human pathogens in most of the world. The reservoir(s) for serogroup O:8, which prevails in the U.S.A., is uncertain (Schiozawa et al., 1991). The pig is the only animal consumed by man which regularly harbors pathogenic Y. enterocolitica. Improved isolation methods and DNA colony hybridization using genetic probes has indicated that the prevalence of pathogenic Y. enterocolitica in pork products is substantially higher than previously suggested. Prevention and control measures should focus on information of people involved in food processing and preparation and on the improvement of hygiene during slaughtering of swine. Important critical control points at the stage of slaughter are: (i) circum anal incision and removal of intestines, (ii) excision of the tongue, pharynx, and particularly the tonsils, (iii) post-mortem meat inspection procedures which involve incision of the mandibular lymph nodes, and (iv) deboning of head meat (Kapperud, 1991). Y. enterocolitica and related bacteria have frequently been isolated from raw milk, but none of the isolates, with the possible exception of serotype 0:5, 21, are recognizable as pathogens. Under normal circumstances Y. enterocolitica does not survive pasteurization. If introduced into pasteurized milk, it can grow well at refrigeration temperatures. Two outbreaks of yersinia have occurred that involved pasteurized milk (Ackers et al., 2000). Pigs, which frequently carry pathogenic Y. enterocolitica in their throat, are the probable source in one of these outbreaks. The most rapid enrichment procedure available for isolation of Y. enterocolitica requires 6 days. No isolation method is available for selective
isolation of pathogenic *Y. enterocolitica* in the presence of related bacteria common in milk and other foods (Schiemann, 1987; Black et al., 1978; Ackers et al., 2000). Chowmein consumption also caused *Y. enterocolitica* gastroenteritis outbreak (Shayegani et al., 1983).

Selective enrichment techniques for *Y. enterocolitica* include cold enrichment and alkali treatment of specimens. Although cold enrichment is effective (Pai et al., 1979; Weissfeld and Sonnenwirth, 1980), it is a time consuming technique and may not allow for completion of the culture within a clinically relevant time frame. Assessments of the usefulness of alkali treatment have produced conflicting results (Ratnam et al., 1983; Weissfeld and Sonnenwirth, 1980). Various selective and differential media for *Y. enterocolitica* have been described (Agbonlahor et al., 1982; Bowen and Kominos, 1979; Dudley and Shotts, 1979; Schiemann, 1979) and compared (Head et al., 1982), and as a result of these reports most laboratories choose cefsulodin-Irgasan-novobiocin (CIN) agar as a selective and differential medium for screening stool specimens for *Y. enterocolitica* where the pathogen produces characteristic red centered bull eye like colonies.

*Y. pseudotuberculosis* strains are biochemically homogeneous (Mollaret, 1961; Wetzler et al., 1970), whereas the biochemical reactions given by strains of *Y. enterocolitica* are quite variable (Bottone et al., 1974; Wauters, 1973; Toma and Lafleur, 1974). *Y. enterocolitica* strains separate into at least two groups when tested by numerical taxonomy (Stevens and Mair, 1973). The species has been divided into four or five bio-groups on the basis of reactions for indole, xylose, esculin and salicin (Wauters, 1973). Several authors have discussed the possibility that strains of *Y. enterocolitica* should be split into two or more species. Among the atypical strains are rhamnose-positive organisms and sucrose-negative organisms. *Y. enterocolitica* strains are known to show positive reaction for catalase, glucose, mannitol, arabinose, maltose, glycerol, levulose, amygdalin, galactose and ribose. Its property of motility at 22 °C and non motility at 37 °C is widely acknowledged. It is known to produce negative reaction for
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oxidase, arginine, lysine, phenylalanine, hydrogen sulphide, KCN, erythritol, etc. (Brenner et al., 1976). Biochemical characterization of Yersinia species by biochemical characterization using API 20E system is widely recognized (Archer et al., 1987) but criticized at the recent times for differentiation at subspecies level for which infrared spectroscopy based method is advocated (Kuhm et al., 2009).

Based on biochemical reactions Y. enterocolitica is subdivided into 6 biotypes namely 1A, 1B, 2,3,4 and 5 (Bercovier et al., 1979; Wauters et al., 1987). In recent years another biotype 6 is also described. This species encompasses three grades of pathogenicity: mostly nonpathogenic strains (biotype 1A), weakly pathogenic strains (biotypes 2 to 6) and highly pathogenic strains (biotype 1B) (Gierczynski et al., 2009). Of these 1B strain which is considered to be highly pathogenic in best of our knowledge is not observed from fecal isolates of Indian patients so far.

Strains of Y. enterocolitica can also be sub divided on the basis of serotypes; with this being the most commonly used typing method for Yersinia. Serotyping is mostly based on LPS surface O antigen and more seldom on H (flagellar) or K (fimbriae) antigen. Since the initial description of Winblad (Winblad, 1967) of eight O antigens, the list has been extended to 76 (Wauters et al., 1991). Certain serotypes (O:3, O:5, 27, O:8 and O:9) are prevalent among human isolates (Bottone, 1997; Wauters et al., 1987). The geographical distribution of highly pathogenic bio-serotype 1B/08 is believed to be restricted in North America. However the same is recently being reported from Poland, Italy, Japan and Germany (Gierczynski et al., 2009; Schubert et al., 2003).

Several methods have been proposed for molecular characterization of Yersinia and related species at genetic level and the matter is recently reviewed (Foley et al., 2009). The methods which are used for molecular characterization of the pathogen are given below.
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Restriction-based methods

1. Plasmid analysis (Iteman et al., 1996)
2. Restriction fragment length polymorphism (RFLP) analysis (Saken et al., 1994)
3. Ribotyping (Iteman et al., 1996)
4. Pulsed-field gel electrophoresis (PGEF) (Saken et al., 1994)

Amplification-based methods

1. Amplification profiling
2. Amplified fragment length polymorphisms (AFLP) (Boghenbor et al., 2006)
3. Random amplified polymorphic DNA PCR (RAPD-PCR) (Williams et al., 1990)
4. Repetitive element PCR (Rep-PCR) (Wojciech et al., 2004)
5. Variable number of tandem repeat (VNTR) analysis and multiple locus VNTR analysis (MLVA) (Gulati et al., 2009)

Sequencing-based methods

1. Multilocus sequence typing (MLST) (Kotetishvili et al., 2005)
2. Single nucleotide polymorphism (SNP) analysis (Price et al., 2007)

Among all the above methods of molecular characterization, PCR based detection of virulence genes of *Y. enterocolitica* is widely used in clinical isolates (Thoerner et al., 2003). PCR amplification of virulence genes of the pathogens which are commonly done are either chromosomal virulence genes (*ystA, ystB*, and *ail*) or plasmid-borne genes (*yadA* and *virF*). 16S rRNA based PCR method is popular among the various methods of PCR identification of the pathogen (Arnold et al., 2004). PCR-RFLP based method is also employed for molecular characterization of *Y. enterocolitica* (Gierczynski et al., 2001). Restriction enzyme analysis of virulence plasmid (REAP) with EcoRI and ribotyping with EcoRV are valuable alternatives to bioserotype determination of *Y. enterocolitica*. Pulse
Field Gel Electrohoresis Typing (PFGE) is the most suitable method of epidemiological tracing of the bacteria (Itten et al., 1996).

Outer membrane proteins (OMP) are one of the key players of bacterial adaptation to host niches and Y. enterocolitica is no exception (Lin et al., 2002). 38kD OMP is proposed to be a biomarker for detection of Y. enterocolitica in the environment or for sero-monitoring (Shin et al., 2002). Expression of the temperature-inducible outer membrane proteins of Y. enterocolitica is observed during infections (Bölin et al., 1985). The major Y. enterocolitica serum resistance determinants include outer membrane proteins yadA and ail of which ail is expressed at normal body temperature at aerobic conditions (Pederson and Pierson, 1995).

In epidemiological studies, differentiation of species into types is necessary to ascertain the prevalence of pathogenic types in a particular region as well as to identify reservoirs of infection, transmission vehicles and routes. To differentiate Y. enterocolitica strains, both phenotyping and genotyping has been used in the present study.

Y. enterocolitica is a recognized causative agent of acute and persistent diarrhea (Nimri and Megdani, 2004; Marks et al., 1980). Although there is a considerable current interest regarding Y. enterocolitica induced diarrhea throughout the globe, only scanty literature is available about the same in the Indian context. The Food borne Diseases Active Surveillance Network (FoodNet) of CDC’s Emerging Infections Program, which collects surveillance data from 10 US states, identified a total of 17,883 laboratory-confirmed cases of infection in 2007. The number of cases and incidence per 100,000 populations reported are as follows: Salmonella (6,790; 14.92), Campylobacter (5,818; 12.79), Shigella (2,848; 6.26), Cryptosporidium (1,216; 2.67), STEC O157 (545; 1.20), STEC non-O157 (260; 0.57), Yersinia (163; 0.36), Listeria (122; 0.27), Vibrio (108; 0.24), and Cyclospora (13; 0.03). The report found that the incidence of Yersinia infections did not change significantly compared with data collected from 2004-2006 (Centers For Disease Control and Prevention, 2008). As in
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developed countries like USA with its improved sanitation is unable to control *Y. enterocolitica* induced gastroenteritis over the years, in the Indian scenario with unachieved sanitation goals the pathogen is expected to cause significant incidence of diarrhea. Therefore it is need of the hour to study *Yersinia enterocolitica* induced gastroenteritis in Indian context. It is believed that the results of such investigations will definitely help to formulate strategies for prevention, control and management of diarrhea in our nation.