Yersinia enterocolitica is recognized as an enteropathogen throughout the globe causing diarrhea and its epidemiological significance is recorded in many international periodicals of repute since long (Napiórkowska et al., 2009; Wang et al., 2009; Zheng et al., 2008; Bobel and Sadkowska-Todys, 2008; Sakai et al., 2005; Singh et al., 2003; Babić-Erceg et al., 2003). Inspite of considerable current interest generated about the pathogen causing diarrhea including diarrhea in the pediatric age group, very little documented research work is available about the pathogen in Indian context. To the best of our knowledge, the highly pathogenic bio-type 1B of Y. enterocolitica has never been reported from India although biotype 1A is reported from stool sample of Indian diarrhea patients (Singh et al., 2003).

In the present study, out of 495 diarrhea stool samples isolated, 6 stool samples showed presence of Yersinia enterocolitica. Out of these, 5 samples were proved to be pathogenic by Congo Red Dye Uptake Test and were classified as highly pathogenic biotype 1B by WHO reference center for Yersinia spp., i.e. Pasteur Institute, Paris. The isolated pathogenic strain was also proved to be diarrhea causing by rabbit ileal loop experiment. These results are further corroborated by histopathological studies where the intestine exposed to isolated virulent bacteria showed altered villus architecture with increased inflammatory cell infiltration. To the best of our knowledge this is for the first time that the highly pathogenic biotype of Y. enterocolitica is being reported from stool samples of patients suffering from diarrhea in India. In corroboration with the report of Pasteur Institute, only the five isolated highly pathogenic strains of Y. enterocolitica biotype 1B were shown to contain ail gene which has been reported to be present only in the highly virulent strains of Y. enterocolitica (Miller et al., 1979). This accounts for the toxicity of the strain at the molecular level.
Discussion

all the eight isolated strains of Y. enterocolitica a 38 kD outer membrane protein was observed to be present. This finding is in consistence with the earlier studies in which the presence of 38 kD outer membrane protein in all strains of Y. enterocolitica has been reported regardless of its virulence (Shin et al., 2002). Apart from this, another novel observation of this work is that, only in the five isolated highly pathogenic Y. enterocolitica strain 1B a 17kD outer membrane protein was observed. This was shown to be absent in the three isolated biotype 1A strains. This particular observation also correlates with the known fact that the expression product of pathogenic ail gene is a 17kD protein that must be present in the highly pathogenic strain of the bacteria alone as ail gene was observed only in the highly pathogenic strain of the bacteria by other workers (Beer and Miller, 1992). Therefore, in addition to the identification of ail gene with PCR identification of presence of ail gene, 17 kD outer membrane protein has the potential to be developed as a virulence biomarker of pathogenic Y. enterocolitica in biological samples (Thoerner et al., 2003). Further investigations are required in this area to establish 17 kD as a biomarker for pathogenic bacteria.

It must be noted by academicians, policy makers, physicians and public at large throughout the globe, that highly pathogenic 1B biotype of Yersinia enterocolitica can cause diarrheal illness in India and so appropriate measures should be taken for the control of the same. We believe that this data is only the tip of the iceberg and wide scale epidemiological survey should be done to evaluate the incidence and prevalence of the highly pathogenic strain in Indian context and to formulate therapeutic/ diagnostic/ prevention strategies to combat human pathogenesis due to the organism; failing which we will not be appropriately prepared to tackle community infection caused by the pathogen in situation of epidemics/ endemics in decades to come.

Y. enterocolitica biotype 1A which is classically known to be nonpathogenic has been documented to cause diarrhea in a considerable number of studies from various part of the globe and India being no exception to this (Singh et al., 2003). In Indian scenario, Y. enterocolitica 1A is also isolated
from diarrheal outbreaks in the community (Abraham et al., 1997). Therefore the pathogenic potential of biotype 1A of the bacterium is established beyond any doubt. Corroborating with the above studies, we have also observed the presence of 1A biotype of the bacterium (which is also classified by Pasteur Institute) in three stool samples collected from diarrhea patients. This further proves the fact that 1A strain of the bacterium can cause diarrhea in humans. 

In spite of the fact that diarrhea is caused by 1A strain of the isolated bacteria, ail gene is not observed to be present in the said strain. This is an accordance with earlier studies which showed that ail gene is absent in 1A biotype of the bacteria. Corroborating the gene expression result, a 17kD outer membrane protein was not observed in the 1A biotype of the isolated bacteria while it was present in the 1B biotype of the isolated strain. Consequently we report here that the protein product of ail gene expression is known to have a molecular weight of 17kD. Both 1A and 1B biotypes showed the presence of 38kD outer membrane protein. This is in support of the earlier observation that 38kD outer membrane protein is omnipresent in all strains of Y. enterocolitica regardless of its virulence. All these observations would help in the development of a specific ELISA test based on 38kD and 17 kD outer membrane proteins which would have the potential to diagnose infection of Y. enterocolitica and further would help in the classification of the bacteria into pathogenic 1A and highly pathogenic 1B strain. Further the ELISA test can be made highly sensitive and specific by the development of monoclonal antibodies to the two proteins.

The biochemical tests revealed that the highly pathogenic 1B strain showed inability to hydrolyze esculin while the 1A biotype of Y. enterocolitica hydrolysed esculin quiet effectively at 33-37 °C when incubated for 24 hours. This shows that esculin hydrolysis test has the potential to be developed into a virulence determination biomarker of Y. enterocolitica. This finding is in partial contradiction with the earlier observation which cautioned about esculin hydrolysis alone to have any sensitivity before at least 48 hours of incubation (Farmer et al., 1992).
So far it is has been reported that both the biotypes (1A and 1B) of *Yersinia enterocolitica* cause diarrheal illness in Indian patients, in and around Chandigarh. The source of this bacterial infection has not yet been reported. To address the issue, we have studied the presence of *Y. enterocolitica* in pork meat, pig throat swab, milk and drinking water, as the infection is described in humans mainly from the above sources. We have observed that pig throat swab contained both 1B and 1A biotype of the bacteria (classified by Pasteur Institute, Paris). From pork meat and milk samples frequently 1A strain of the bacteria is isolated. From drinking water the bacteria was not isolated.

The earlier studies are frequently advocating presence of highly pathogenic 1B strain of the bacteria in pig which are also *ail* gene positive (Fredriksson-Ahomaa *et al.*, 2007). Pork meat is also known to contain 1A strain of the bacteria. Milk and its products are also known for presence of the bacteria (Schiemann, 1987; Black *et al*., 1978; Ackers *et al*., 2000). Our observations are in corroboration with these described facts in the literature and there is every possibility that infection to the human host occurred from pig/pork products and milk/milk products in the described patients although absence of such cause and effect analysis is one of the limitation of the work. Although contaminated water is a known infecting agent of the bacteria and water born infection of *Y. enterocolitica* is described in Indian context, we have failed to isolate the bacteria from drinking water in and around Chandigarh. Therefore we are not in a position to highlight about this specific cause and effect association regarding the infection as a cause of diarrhea in the described patients. Further studies are required to investigate such an association.

The main achievement of this work is that it has isolated 1B highly pathogenic strain of *Y. enterocolitica* from stool of Indian diarrhea patient for the first time to the best of our knowledge. The strains are currently being maintained by Pasteur Institute, Paris. There are many limitations of the study. Firstly within the limited resource available for the study and ofcourse maintaining the inclusion and exclusion criteria as strictly as possible, we are unable to screen more than 495 patients. Secondly, as we have worked mostly in the slum areas a
follow up of the patient population studied was not possible. Thirdly a real cause and effect analysis of the diarrhea is not done. To understand about detail of these epidemiological aspects future studies on the subject must be done by various centers located at various parts in India.

From the extent of work done we hypothesize that pork product is the potential source of infection by highly pathogenic *Y. enterocolitica* 1B biotype in Indian subcontinent analogous to any part of the globe. However pork or pork products and contaminated milk or milk products are the potential sources of infection caused by 1A biotype of the strain in the Indian context. It is also suggested that in the culture of stool samples for determination of causative agent for diarrhea, the laboratories must keep the basic infrastructure to diagnose *Y. enterocolitica* and should not suppose blindly that the infection is very rare in India and so should not checked for *Y. enterocolitica*

We further conclude that *Yersinia enterocolitica* must not be ignored as a pathogen causing diarrhea in Indian subcontinent but should be regarded as a potential pathogen and extensive research should be carried out to understand epidemiology of bacteria induced gastroenteritis in India.