Chapter 6
Summary and Conclusions

Yersinia enterocolitica is a recognized causative agent of acute and persistent diarrhea. 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. Therefore, the present study was carried out with an aim to isolate strains of Yersinia enterocolitica from stool samples of diarrheal patients in and around Chandigarh and to investigate the prevalence and epidemiological importance of the bacterium.

Sample Collection, Colony Identification and Confirmation

495 stool samples were collected from individuals who were suffering from diarrhea in and around Chandigarh. The samples were cultured on MA, XLD & SS agar. The colony characteristics were compared with the control strains of Yersinia enterocolitica & Salmonella typhimurium. Out of 495 samples, 252 samples were observed to contain bacteria belonging to family Enterobacteriaceae. These samples were subjected to cold enrichment and alkali treatment. These typical colonies of 352 samples were then tested by Gram staining. Typical colonies of 285 samples observed to contain Gram negative bacilli while rest harbored Gram positive bacteria. Since Yersinia is gram negative, the 285 samples Gram negative bacterial colonies were then subjected to catalase and oxidase test. All the gram negative bacteria showed positive catalase test and negative oxidase test and therefore, they were included in Enterobacteriaceae family. To identify Yersinia enterocolitica out of these 285 samples, urease and motility tests were performed. 8 samples showed positive urease test and motility at low temperature. Yersinia enterocolitica was observed to be motile at 22°C and non-motile at 37°C. Thus 8 samples contained Y. enterocolitica.
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To further confirm the bacterial species, a number of biochemical tests were carried out. *Yersinia enterocolitica* was urease producing, non hydrolyzing lysine but hydrolysing ornithine. All isolated strains were also observed to be indole positive. However, the esculin hydrolysis capacities of strains were variable. From 8 samples observed to contain *Yersinia enterocolitica*, 5 samples were unable to hydrolyse esculin. These samples harbored the pathogenic strain of the bacterium and the rest 3 samples contained the non-pathogenic strain of the bacterium. These results were proved by Congo red dye uptake test. Pathogenic bacteria gave positive test and non-pathogenic bacteria gave negative test.

All the isolated pathogenic and non pathogenic *Yersinia enterocolitica* strains showed characteristic growth on CIN, Blood and Nutrient Agar. The pathogenic and nonpathogenic *Yersinia enterocolitica* isolated from samples have showed similar colony characteristics.

Biotyping and Serotyping

To further confirm the pathogenicity of the isolated bacterial strains, the samples were sent to Pasteur Institute, Paris, the referral centre for *Yersinia* where biotyping and serotyping were done. All the 3 suspected pathogenic *Yersinia enterocolitica* isolated from human stool samples were confirmed by Pasteur Institute, Paris. They were reported to belong to biotype 1B & serotype 7, 8-8-13-8, 19. The non-pathogenic strain of *Yersinia enterocolitica* was classified into biotype 1A & serotype 41, 42-41, 43.

Molecular Characterization

Molecular characterization of the isolated strains was done by PCR and SDS-PAGE.

PCR: All the 8 isolated strains showed the presence of 16s rRNA gene irrespective of the pathogenicity. The 5 pathogenic strains showed the presence of *ail* gene. The gene was not observed in the non-pathogenic strain.
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**SDS-PAGE:** The 17kDa outer membrane protein was present in all the 5 pathogenic strains of *Y. enterocolitica* and absent in the non-pathogenic isolates. However, a 38kDa outer membrane protein was observed in all the isolated *Yersinia enterocolitica* strains irrespective of pathogenicity.

**Isolation and Characterization of *Y. enterocolitica* from pig throat swabs**

22 pig throat samples were collected and studied for the presence of *Y. enterocolitica*. Out of the 22 samples, three isolated strains belonged to *Y. enterocolitica* as proved by catalase test, oxidase test, motility test and urease test. Similar to the human samples, these samples were also analyzed for the presence of pathogenic and non-pathogenic *Y. enterocolitica*. Two isolates proved to be pathogenic and one non-pathogenic by esculin hydrolysis test and Congo red dye uptake test. These results were further confirmed by Pasteur Institute, Paris.

**Rabbit Ileal Loop Test**

The pathogenicity of the isolated pathogenic strains was cross-verified by rabbit ileal loop test. Intestinal loops injected with pathogenic bacteria were observed to be dilated due to accumulation of fluid while those injected with non-pathogenic bacteria were not dilated.

**Histopathological Studies**

The sections of the rabbit intestine injected with pathogenic *Y. enterocolitica* showed damaged villi and inflammatory cell infiltration. While the intestinal sections exposed to non-pathogenic bacteria were observed to be normal.

. The present study provides an insight into the prevalence of *Y. enterocolitica* in and around Chandigarh. Further studies are required to control the bacterial infection in this area.