# ABSTRACT

The firts step in the pathogenesis of *Vibrio cholerae* (*V. cholerae*) infection is its adherance on enterocytes. Enterobacteriacea are known to used pili proteins or Outer membrane proteins (OMP-s) as adhesion molecules. The purpose of this reseach was to study the adhesion molecules used by *V. cholerae* to adherence to enterocytes using *Vibrio cholerae* O1 M094V and rat's small intestine as models. *Vibrio cholerae* O1 M094V used in this study was originally obtained from fecal isolates of patients treated at Saiful Anwar General Hospital Malang.

Pili proteins were isolated occording to modified Ehara method. In this procedure, bacteria were first grown in TCBS (Thiosulfat Citrat Bile salt Sucrose Agar) culture medium, and transfered to TCG ( Thioproline Carbonat Glutamat) medium, which is known to enhance pili formation. Bacterial pili were then sheared in two stages, collected, run on SDS-PAGE (Sodium Dodecyl Sulfat Polyacrylamid Gel Electrophopresis), and individual pili proteins isolated by electroelution. Bacterial Omp-s were obtained occording to modified Evav's method using NOG (n-Octyl-β-D-Glucopyranoside).

Enteocytes were obtain from rat's intestine occording to the method of Weisler. Enterocyte membrane proteins were then isolated using the same method as was used to isolate bacterial Omp-s.

Using the above methods, the following proteins of different M.W-s were obtained, i.e. : (a) pili proteins: 50.3 kDa., 37.8 kDa., 35.9 kDa., 21.3 kDa., 16.6 kDa. and 10.5 kDa.; (b) bacterial Omp: 37.8 kDa.; (c) enterocyte membrane proteins: 62 kDa., 28.9 kDa., 12.7 kDa. and 10 kDa. All pili proteins , except the 16.6 kDa. and 10.5 kDa. proteins, showed hemagglutinin activities. The 10.5 kDa. protein showed anti-hemagglutination activity, whereas the 16,6 kDa. protein showed neither hemagglutinin nor anti-hemagglutinatinin activity.

The question now arises as to which bacterial protein act as adhesion molecules, and which enterocyte membrane proteins act as their receptor. To addres this problem, three series of adhesion studies were done. The firt series involeved adhesion of free (uncoated) bacteria to enterocyte coated with the putative adhesion molecules. The second series involved adhesion of bacteria coated with either pre-immune chicken yolk sac IgG ( IgG Y) or immun IgG Y against 37.6 kDa. Omp to free enterocyte and the third series involved adhesion of bacteria coated with the putative receptor molecules to fre enterocytes.

By cakculating the adhesion index (AI) and regresion analysis of the relationship between AI and different concentration of the coating molecules, it could be concluded that:

1. the 37.8 kDa. Omp and all pili proteins except the 16.6 kDa. protein can act as adhesion molecules.
2. The 62 kDa, 12.7 kDa.and 10 kDa. enterocyte membrane proteins are receptors for the bacterial adhesion molecules.

Evidence was provided that the 37.8 kDa. Omp in its natural form has a M.W aproximately twice as high (75,2 kDa.). It is also suggested that the pili adhesion molecules probably exist as a polymer of the 10.5 kDa. pili protein.

**Key words**: *Vibrio cholerae* O1 M094V, hemagglutinin, pili protein, Outer membrane protein (OMP), adhesion molecule, enterocyte receptor.