45 kDa FIMBRIA PROTEIN OF *Proteus mirabilis* AS HEMAGGLUTININ AND ADHESION PROTEIN

Diana Chusna Mufida[1], Enny Suswati[1], Septa Surya Wahyudi[2], Agung Kurniawan[3]
[1]Laboratory of Mikrobiologi Faculty of Medicine University of Jember
[2]Laboratory of Patologi Klinik Faculty of Medicine University of Jember
[3]Laboratory of Anatomi Faculty of Medicine University of Jember

ABSTRACT

Proteus mirabilis is opportunistic and nosocomial pathogen that usually found in clinical specimen from patients with catheter. The pathogenic mechanism of the bacteria are not fully elucidated especially its potential activity of the protein as hemagglutinin and adhesion molecule. The aim of this study is to evaluate the role of 45 kDa fimbria protein from P. mirabilis. After identification, bacterial isolate of fimbria fraction 12,5 % SDS-PAGE were used to isolate fimbria protein followed by hemagglutinin test and invitro adhesion test. The study showed that the 45 kDa fimbria protein of P mirabilis was a hemagglutinin protein that could agglutinate mice erythrocytes, rabbit erythrocytes, and human group O erythrocytes. Hemaglutination test were negative on erythrocytes human blood group A,B, and AB. The 45 kDa fimbriae protein was also adhesion protein showed by its activity to adherence to rabbit vesica urinaria epithel receptor. The increase dose of 45 kDa fimbriae protein will decrease the mount of P mirabilis bacteria to adherence to rabbit vesica urinaria epithel (p< 0,05).

Keywords**:** *Proteus mirabilis, fimbriae, hemagglutinin protein, adhesion protein*

Correspondence: Diana Chusna Mufida, Laboratory of Mikrobiologi Faculty of Medicine University of Jember, Jl. Kalimantan 37, Jember, phone (0331)337877, email : chusna2005@telkom.net

INTRODUCTION

Proteus mirabilis is one of the most important causes of urinary tract infection (UTI). UTI caused by these bacteria are persistent, difficult treatment, and can be fatal. *P mirabilis* produces urease which splits urea into ammonia and CO2, which will increase the pH of urine. With a pH of urine increased the precipitation components (Ca^{2+} and Mg^{2+}) to more easily giving rise to the formation of stones. The existence of stones in both kidneys and urinary vesica cause urinary tract infections become persistent and recurrent. In addition to the stone and infection together will result in kidney damage, either acute or chronic pyelonephritis and can also cause bacteremia (Perepelov et al L999, Wassif et al L995). In addition to the invasion of *P mirabilis* and settled in the epithelial cells of the epithelial cells, causing these bacteria become difficult to treat with some types of antibiotics such as cotrimoksasol, amoxycillin - clavulonat acid, nitrofurantoin, and ciprofloxacin (Mathoera 2002).

Pathogenesis of bacteria to cause a disease, in general there are two stages. In the first stage will make sticking to the bacterial host cell, in the initial adhesion and nature played by pili anchoring adherence is then followed by adhesion through the outer cell membrane, which adherence is doching. After doing sticking the bacteria will multiply along with the production of materials that can be detrimental to bacterial metabolism of host cells (Salyer & Whitt 2002). Adhesin molecule is a bacterial virulence factor and in some bacteria hemagglutinin protein functions as an adhesion protein (Salyer & Whitt 2002, Noorhamdani 2005). Molecular weight varies between a bacterial adhesin with other bacteria. *Acinobacter baumanni* have hemagglutinin molecule and is also an adhesin molecule (Noorhamdani 2005). Likewise, the bacterium *Salmonella typhi* has a hemagglutinin molecule that also acts as an adhesin, pili proteins with molecular weights of 36 kDa (Sanarto 2002). The purpose of this study is to prove the existence of haemagglutinin protein that functions as an adhesin, particularly fimbria 45 kDa protein from the bacterium *P. mirabilis.*

MATERIALS AND METHODS

P. mirabilis Identification

An aseptic specimens grown on Mac Conkey agar medium and incubated at 37 oC for 18-24 hours.

Colonies grew Gram stain was made and viewed under a microscope. Identification of bacteria was done by microscopic examination of bacterial rod shape or kokobasil. When cultured on agar medium will appear to the phenomenon of swarming. Test phenilalanin +, urease +, H2S +, ornithin +, indole-,-fermented adonitol, inositol -.

Subcultures of *P. mirabilis*

Bacteria to be used is P mirabilis strains from a local patient urine bacteriuria, method used as prescribed by Ehara (1992), ie growth media that enrich TCG pili P mirabilis. This media thioproline Containing 0.02%, 0.3% NaHCO3, 0, l5 bactotrytonr, 0.2% yeast extract, 0.5% NaCl, 2% bacto agar and 1 mm EGTA. Media to be made in the capacity of 250 ml bottles are tilted as much as 50 ml of agar. P mirabilis are grown on Brain Heart Infusion (BHI) are incubated at 37oC for 4 hours. Then as many as l0 ml suspension of bacteria included in each bottle containing TCG media. Furthermore, incubation performed at a temperature of 37^{o}C for 2x24 hours.

Isolation of *Proteus mirabilis* Fimbria

Fimbria harvested from 50 bottles of cultured bacteria. Results collection chlore bacteria added tri acetic acid (TCA) to 3% concentration. Having beaten laid flat at room temperature for l hour and every l5 minutes in shakes. Furthermore, centrifuged 6000 rpm for l0 minutes at a temperature of 4 oC. Pellets were taken and resuspensized with PBS pH 7.4 solution with a ratio l: l0. Bacteria own shaved using an electric mixer. Bacteria shaved full speed for l minute, repeated up to five times with l-minute rest period. The result is centrifuged for 30 minutes at 4oC l2000 rpm. Fimbria which is located at the top was taken. Solution and suspended sediment same way as above and collected in a way to shave again until a couple of times, until the resulting supernatant which shows a negative agglutination test or turbidity with PBS (Ehara 1992).

Sodium Dodecyl Sulfate Gel Electroforesis Poliacrilamide (SDS-PAGE)

Monitoring molecular weight was performed using SDS-PAGE (Smeds et al. 2001). L00oC protein samples heated for 5 minutes in a buffer solution containing 5 mm Tris HCl pH 6.8, 2 - mercapto ethanol, 5%, w/v Sodium Dodecyl sulfate 2.5%, v/v l0% glycerol with bromophenol blue tracer color. Selected mini-slab gel l2, 5% by tracking the gel 4%. L25 mV voltage used. The materials used are coomasive brilliant blue and low sigma range of standard molecular markers.

Fimbria Protein Purification Fractions

The method has been performed as a modification done by Ehara (Sumarno 2000). The SDS-PAGE gel fimbria collection, straight cut its gel desired molecular weight and a piece of tape is to be collected and included in the membrane dialysis fluid using electrophoresis buffer, running buffer. Electrophoresis is then performed using flow electrophoresis apparatus frontal l25 mv for 25 minutes. The result of electrophoresis performed dialysis fluid with PBS buffer pH 7.4 for 2x24 hours @ l liter and replaced two times. Dialysate fluid was performed hemagglutination test.

Hemagglutination Test

Hemagglutination test was done according to instructions from Li (l999). Concentration of sample dilution was made l/2 on microplate V, where each 50th µl volume wells. Each well added a suspension of red blood concentration of 0.5% volume mice were the same. Then shaken with rotator plate for l minute. Subsequently placed in room temperature for l hour. The amount of the titer determined by observation of red blood agglutination at the lowest dilution. The samples tested were crude pili, pili proteins, protein OMP Proteus mirabilis. Blood that is used is the blood of mice and rabbits and humans.

Epithelial Cell Isolation Urinary Rabbit Gallbladder

Isolation of epithelial cells of the rabbit urinary vesica performed according to the method Weisser (Nagayama et al.1995). Rabbits used is a healthy rabbit with approximately 1.5 kg. Rabbits are anastezied using chloroform, and then taken part vesica urinary cut and opened. Gallbladder urinary washed with PBS pH 7.4 containing 1 mm dithiothretiol at 4oC until they are clean. After it was inserted in the gallbladder urinary fluid containing 1.5mm KCl, 6.9 mMNaCl, 27 mMNa Citrat, 8 mM and 5.6 mM Na2HPO4 KH2SO4 with a pH of 7.4, then incubated in shaking incubator network for 15 minutes, with a temperature of 37^{o}C. Supernatant discarded and the tissue removed in the liquid containing 1.5 mm EDTA and 0.5 mM dithiothretiol. Furthermore, a network that is in fluid containing EDTA and dithiotretiol was shaken strongly for 15 minutes at a temperature of 37^{o}C, and then supernatant discarded. Network washed with PBS and centrifuged for 5 minutes at 1000rpm, and was repeated 3 times. Gallbladder epithelial urinary isolated by suspension in the network by using sterile PBS and subsequently calculated with 560nm wavelength spectrophotometer until the concentration of 106/ml. Vesica urinary epithelium is ready to test adhesion.

Adhesion Test

Adhesion test modifications Nagayama (l995), the adhesion test Proteus mirabilis was cultured in lactose broth at a temperature of second bacteria harvested by using centrifugation 6000 rpm for l0 minutes at 4oC. Sediment suspended with PBS and bacterial content of l0 8/ml made using spectrophotometer with a wavelength of 600nm. Furthermore, the protein dose fimbria preparation made of each of 0µl (dick), 25 µl, 50 µl, 100 µl µl, 200 µl, 400 µl and 800 ul in 300 µl PBS. Furthermore, each dose added to a suspension of 300 µl epithelium and shaken slowly at shaking waterbath at a temperature of 37^{o} C for 30 minutes. Then in each mixture is added 300 µl of bacterial suspension. Mixtures were incubated with shaking incubator for 30 minutes at a temperature of 37^{o} C. Subsequently centrifuged at 1500 rpm and 4^{o}C for 3 minutes, then sediment washed two times with PBS. Then the deposition was taken and made smear on glass objects and painted with Gram stain. Preparations were observed with 1000 times magnification microscope and counted the number of bacteria that adhere epithelial cells. Adhesion index is the average number of bacteria that adhere on the epithelium, is calculated for each observation on 100 epithelial (Martino et al. 1995).

Statistical Analysis

Statistical analysis using ANOVA and regression testing, with a limit of 0.05 significant.

RESULTS

After the identification of *Proteus mirabilis* from the urine of UTI patients using Microbach, then subsequently the bacteria on biphasic culture media, TCG-enriched BHI for growth of fimbria. After 48 hours the bacteria are harvested and made cuts fimbria. After the cutting of pili in stages, until the color of the supernatant pieces fimbria, hemagglutination test with the following results, as shown in Table 1.

Table 1. Fimbria hemagglutination test various pieces of *Proteus mirabilis* by dilution rise in erythrocytes of mice

Pieces of Fimbria	Dilution									
	1x	2x	3x	4x	5x	6x	7x	8x	9x	10x
1	+	+	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	-	-	-
3	+	+	+	+	+	+	+	-	-	-
4	+	+	+	+	+	+	+	-	-	-

Hemagglutination test results showed that pieces of the fimbria-2, 3, and 4 showed the same titer, ie up to -7 dilution still capable hemeaglutinate erythrocytes in mice. Further pieces of fimbria SDS-PAGE performed to predict the molecular weight of proteins, with results as shown in Figure 1. Protein profile on SDS-PAGE of several pieces of *Proteus mirabilis* fimbria showed a prominent protein with a molecular weight proteins 45 kDa, 35 kDa, 23kDa and 20 kDa. Furthermore, these truncated proteins that stand out and be done Electrophoresis and dialysis, the protein solution thus obtained. From the results of electro-elution and dialysis are protein fimbria hemagglutination test in erythrocytes of mice, rabbits, human blood groups A, B, O, AB and the results are shown in Table 2.

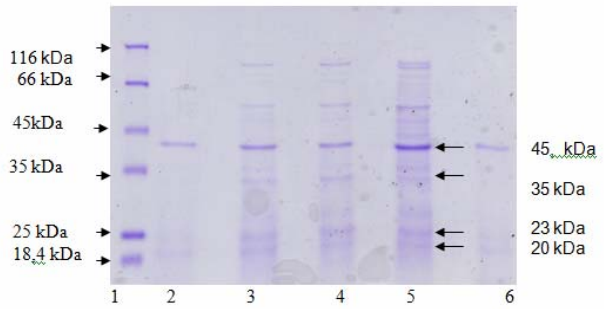

Figure 1. The SDS-PAGE and various pieces of fimbria P mirabilis. Wells 1, the protein tracer, two pieces of pili first wells, three wells into two pieces pili, pili three wells four pieces, five pieces of pili four wells

Table 2. Hemagglutination test of Proteus mirabilis fimbria proteins 45 kDa molecular weight with dilution rise to various types eritosit

Erythrocyte Sample	Dilution									
	1x	2x	3x	4x	5x	6x	7x	8x	9x	10x
Mouse	+	+	+	+	+	+	+	+	+	-
Rabbit	+	+	+	+	-	-	-	-	-	-
Human O	+	+	-	-	-	-	-	-	-	-
Human A	-	-	-	-	-	-	-	-	-	-
Human B	-	-	-	-	-	-	-	-	-	-
Human AB	-	-	-	-	-	-	-	-	-	-

After the adhesion test of hemagglutinin proteins with different molecular weights obtained images of bacteria attached to epithelial cells in rabbit urinary vesica considerable amount, as shown in Figure 2. We performed adhesion inhibition test using fimbria protein with molecular weight of 45 kDa obtained results of a decreasing number of bacteria that attach to the urinary epithelium vesica compared with non-coated with these proteins, as shown in Figure 3. While the average test results of adhesion on the rabbit urinary epithelium vesica overlaid or not overlaid F 45 kDa protein with a dose-storey, from dose of 100 µl to 12.5 µl is shown in Table 3.

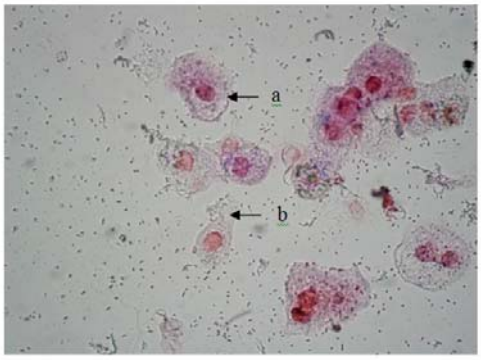

Figure 2. Proteus mirabilis adhesion on the epithelium of the rabbit urinary vesica. Arrows indicate a rabbit urinary epithelium vesica, b shows the bacteria *P. mirabilis*

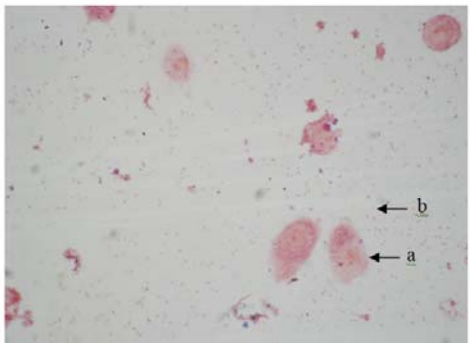

Figure 3. Proteus mirabilis adhesion previously been overlaid with frimbria 45 kDa protein with a concentration of 200 µl. Arrows indicate epithelial vesica urinary a rabbit, b shows the bacteria *P. mirabilis*

Table 3. Average test results in epithelial adhesion Proteus mirabilis urinary vesica rabbits using 45 kDa protein F

Protein Dose	Adhesion index
0	1113,33
25	832
50	624
100	546
200	340
400	284,33
800	160

From Table 3, the description of a decreasing number of bacteria attached to epithelial urinary vesica after overlaid with M 45 kDa protein. And a decrease in the number of bacteria attached to epithelial along with the increasing doses of F 45 kDa protein.

Results from this study, then made a linear regression statistical test results of 0.637 and R 0.762 R2. While the ANOVA results obtained α 0.03. Statistical test results indicate that there are F 45kDa protein dose effect on the number of bacteria attached to epithelial vesica urinaria. There was also significant difference between the amount of bacteria on the epithelium vesica urinary melakat that are not overlaid with protein F 45kDa with the number of bacteria attached to epithelial vesica urinary proteins that have been overlaid with F45kDa.

DISCUSSION

Proteus mirabilis included in the family *Enterobacteriaceae*, in the process of the inception of the disease through the adhesion process is played by the fimbria and OMP. Success of infection was strongly influenced by the ability to perform adhesion. In this study, conducted tests on protein adhesion fimbria. First step is to do the research culture of Proteus mirabilis on media biphasic TCG-BHI with the aim to grow the bacterial fimbria optimally. After it was done in stages fimbria withholding, until the supernatant turbidity cuts have the same fimbria with PBS. Cutting result fimbria hemagglutination test against red blood cells. At this stage hemagglutination test using rabbit red blood cells. From the results obtained by hemagglutination test, all the pieces of Proteus mirabilis fimbria able hemeaglutinate red blood cells up to dilutions of 2x on the first piece, and diluting 7 x on the snippet of the fimbria-2 until the number-four.

The next step was to determine the molecular weight contained in the fimbria. To identify proteins contained in the SDS-PAGE performed fimbria. Based on the results of SDS-PAGE, in Figure 1, can be seen fimbria *P. mirabilis* dominant protein with molecular weight of 45 kDa, 35 kDa, 23 kDa and 20 kDa. Hemagglutination test based on results obtained fimbria subunit, protein subunits with molecular weight 45kDa able hemeaglutinate red blood cells of mice up to 9x dilution, rabbit up to 4x dilution, and human blood type-O until the 2x dilution. Fimbria ability agglutinate red blood cells there are two types, namely mannose resistant (MRHA) and the mannose-sensitive hemagglutination (MSHA). MRHA MSHA will turn into red blood cells when given Tannates acid 0.01%. Hemagglutinin protein can be derived from the fimbria bacteria and/or OMP. Structures that play a role in bacterial adhesion pili or fimbria is composed of a protein called pilin (-fimbrial adhesin) or afimbrial adhesion is a protein found on the bacterial cell surface (Salyer & Whitt 2002). Adhesin on some form of bacterial proteins that can agglutinate erythrocytes proteins known as hemagglutinin (Nagayama et al1995).

Hemagglutinin protein, the ability to agglutinate red blood cells of animals and humans are not the same (Winarsih et al1997, Santoso 2002). In this study, variation was found, the protein is able hemeaglutinate fimbria F 45 red blood cells of rabbits, mice and human blood group O. The data showed that the hemagglutination activity of Proteus mirabilis bacteria differ from other bacteria. Campylobacter pylori hemagglutination activity against erythrocytes of mice, rabbits, guinea pigs, sheep, horses, and humans produce a positive reaction (Nakazawa et al 1989). Acinobacter have fimbria proteins capable hemagglutinated F 16 mice and human erythrocyte blood group O, but cannot hemagglutinated erythrocytes of sheep, guinea pig, rat and human blood groups A, B (Noorhamdani 2005).

Vibrio cholera O1 M094V has fimbria and OMP proteins each of molecular weight 38kDa able to agglutinate erythrocytes of mice, rabbit and human blood type O and could not able to agglutinate guinea pig and human erythrocyte blood groups A, B, and AB (Sumarno 2000). Bacterium *Salmonella typ*hi has a hemagglutinin protein OMP fimbria and each with a molecular weight of 36 kDa able to agglutinate

erythrocytes of mice, guinea pig and human blood type O, but not able to agglutinate sheep erythrocytes, human blood groups A, B and AB (Santoso, 2002).

Proteus mirabilis fimbria proteins have the ability to agglutinate human erythrocytes type O blood, so too *Acinobacter, Salmonella typhi and Vibrio cholera* O1M094V showed that blood group O erythrocyte surface receptors that are complementary and could bind specifically with the adhesin of various types of microbes, particularly Gram-negative bacteria. Since the receptors on the erythrocyte surface receptors similar to the other host epithelial cells, especially epithelial cells that become places of colonization, it is predicted human blood group O in erythrocytes and cell surface receptors epitel has matches and complements the adhesin microbial pathogens compared with the human blood groups A, B , and AB, so that people with type O blood is more sensitive to microbial infections, including Proteus mirabilis. The empirical evidence supported by facts stated that the incidence of ulcer disease caused by Helicobacter pylori increases in human blood group O (Robertson et al. 2003). Besides human blood type O are more sensitive to fungal infections *Pityrosum ovale* (Shankar et al.2002) and Norwalk virus infections often attack people with blood type O and show there is a relationship between genetic factors and the risk of viral infection, Norswalk (Hutson et al 2002).

Next step of this research is to identify the adhesin protein, the adhesion test was done using the hemagglutinin protein weight 45 kDa. In Figure 2 looks a lot of bacteria that attach to the epithelial cells of the rabbit urinary vesica and subsequently in Figure 3, fewer attached bacteria when compared with Figure 2. From table 3 it appears that the greater the dose of 45 kDa protein that coated by hemeaglutinin to vesica urinary epithelium of rabbits, the fewer bacteria attached.

This is caused by Proteus mirabilis receptors on rabbit epithelial uriaria vesica already saturated by fimbria proteins with molecular weights of 45 kDa. The more receptors that saturated by the hemagglutinin protein, the bacteria that stick to the less. From the adhesion test was obtained by empirical results that the pili protein subunits with molecular weight is 45kDa protein adhesion. These results are in accordance with research conducted on Vibrio cholera O1M09V Sumarno, that the hemagglutinin protein OMP pili and adhesion proteins. Similarly, the F protein Acinobacter 16th baumannii is haemagglutinin protein that also acts as an adhesion protein (Noorhamdi 2005).

CONCLUSIONS

Has been proven bacterial *P. mirabilis* isolates from urine of patients with urinary tract infections, has the fimbria proteins with molecular weights of 45 kDa which is the hemagglutinin protein and also acts as an adhesin molecule and is believed as one of the virulence factors. Although further research is still needed, hemeaglutinin adhesin protein molecules can be developed as candidate vaccines in preventing infection.

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