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Molecular Detection of Fimbrial Genes of *Proteus vulgaris* isolated from Patients with Urinary Tract Infection

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Abbreviations:
NAFs: Non-agglutinating fimbriae, also known as uroepithelial cell adhesin (UCA) fimbriae, MRK: mannose-resistant *Klebsiella*-like fimbriae, MRP: mannose-resistant Proteus-like fimbriae, PMFs: *P. mirabilis* fimbriae, ATF: ambient-temperature fimbriae; RBC: Red Blood Cell; CFA: Colonization factor antigen

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Abstract
Proteus vulgaris has been described as an etiological agent in urinary tract infections. *Proteus*strains must attach to uroepithelial cells and the catheter surface to colonize and initiate UTI and may express a variety of adhesins to assist in this initial attachment. In this study, a collection of *Proteus vulgaris* isolates obtained from patients with urinary tract infections and then they tested to detect their having of adhesive factors genes. The infectious trait of this bacterium depends on colonization of urothelial cells. Fimbirae play the main role in this junction. Four kinds of fimbrial genes are more important and responsible for the pathogenicity and the attachment of *Proteus vulgaris* to urinary tract epithelium (*mrp*, *mrkA*, *uca* and *atf*). All fimbrial genes were detected in 100% of isolates.The results obtained in this study indicates that the primers targeting the fimbrial genes are very specific and that the prevalence of adhesive factors genes may correspond to the opportunistic nature of the organism.

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1. Introduction

Bacterial adhesion onto mucosal or urothelial cells is an important phenomenon determining bacterial virulence. Infection in the urinary tract is related in part to the ability of bacteria to adhere and colonize the gut, perineum, urethra, bladder, renal pelvicalyceal system, and renal interstitium (Oelschlaeger et al., 2002). Adhesion is particularly important when infection occurs in an anatomically normal urinary tract, but it is also important in recurrent cystitis and infection complicating indwelling bladder catheters (Nielubowicz and Mobley, 2010). Five fimbrial types have been characterized and reviewed for *Proteus* rods (Rocha et al., 2007). Non-agglutinating fimbriae (NAFs; also known as uroepithelial cell adhesin (UCA) fimbriae, mannose-resistant *Klebsiella*-like (MRK) fimbriae, mannose-resistant *Proteus*-like (MRP) fimbriae, *P. mirabilis* fimbriae (PMFs)and ambient-temperature fimbriae (ATF) (Jacobsen et al., 2008; Chelsie and Harry, 2012).

1.1 Mannose-resistant Proteus-like (MR/P) fimbria

The mannose-resistant *Proteus*-like (MR/P) fimbria are perhaps the best-understood fimbriae expressed by *Proteus* strains during UTIs. These fimbriae are thick channeled (7 to 8 nm) and are classified as mannose-resistant hemagglutination (O'May et al., 2008). These fimbriae assemble through the chaperone-

usher pathway (O'May et al., 2008). The genes required for the expression of MR/P fimbriae on the cell surface are encoded on the *Proteus* chromosome on two divergent transcripts, *mrpABCDEFGHJ-* (designated the *mrp* operon) and *mrpI* (Jacobsen *et al*., 2008). Some of the proteins encoded by the *mrp* operon include the fimbrial structural subunit *MrpA*; the terminator for fimbrial assembly, *MrpB*; the minor fimbrial subunit *MrpG*; the tip adhesion *MrpH*; and the repressor of flagellin synthesis, *MrpJ* (Jansen et al., 2004; Jacobsen et al., 2008). Like that for many other virulence factors, the expression of MR/P fimbriae undergoes phase variation and elicit a strong immune response during infection (Jansen et al., 2004). It is directed against the MrpA protein. Indeed, this type of fimbriae was recently shown to be the best immunogen in terms of conferring protection after immunization (Jansen et al., 2004; Scavone et al., 2011).

1.2 Mannose-resistant Klebsiella-like (MR/P) fimbria

The expression of type 3 fimbriae has been described from many Gram-negative pathogens (Burmolle et al., 2008). Type 3 fimbriae are 2-4 µm wide and 0.5-2 µm long surface organelles that are characterized by their ability to mediate agglutination of tannic acid-treated human RBC (Mannose-resistant *Klebsiella*-like agglutination) (Ong et al., 2010). Several studies have clearly demonstrated a role for type 3 fimbriae in biofilm formation (Burmolle et al., 2008 and Ong et al., 2009). Type 3 fimbriae also mediate various adherence functions such as binding to epithelial cells (from the respiratory and urinary tracts) and extracellular matrix proteins (e.g. collagen V) (Ong et al., 2010).Type 3 fimbriae belong to the chaperone-usher class of fimbriae and are encoded by five genes (*mrkABCDF*) arranged in the same transcriptional orientation (Ong et al., 2010). The *mrk* gene cluster is similar to other fimbrial operons of the chaperone-usher class in that it contains genes encoding major (*mrkA*) and minor (*mrkF*) subunit proteins as well as chaperone- (*mrkB*), usher- (*mrkC*) and adhesin- (*mrkD*) encoding genes (Huang et al., 2009). A putative regulatory gene (*mrkE*) located upstream of *mrkA* has been described previously in *Klebsiella pneumonia* (Ong et al., 2010). The *mrk* genes have been shown to reside at multiple genomic locations, including the chromosome, on conjugative plasmids and within a composite transposon (Norman et al., 2008).

1.3 Uroepithelial cell adhesin (UCA) fimbriae

Uroepithelial cell adhesin (UCA) was initially discovered in anuropathogenic isolate of *P. mirabilis* (strain HU1069) during a screen designed to identify outer membrane proteins that facilitated binding to uroepithelial cells (Rocha et al., 2007). The identified UCA protein, the major structural subunit later designated *ucaA* was purified to homogeneity for characterization; the purified protein retained the ability to bind to uroepithelial cells and, additionally, organized into long, flexible filaments with a diameter of 4 to 6 nm, consistent with the appearance of fimbriae (Nielubowicz, 2010).Subsequent determination of the sequence of the *ucaA* gene revealed that *ucaA* has the highest similarity to F17 and F111 fimbriae from bovine enterotoxigenic *E. coli* (Nielubowicz, 2010). These findings led to postulation that UCA may function as a primary adhesin for *Proteus* in the intestinal tract, although this hypothesis has not been experimentally tested (Cook et al., 1995). UCA fimbriae have since been renamed non-agglutinating fimbriae (NAF) to distinguish them from other *P. mirabilis* fimbriae that contribute to adherence (Tolson et al., 1995 and O'May et al., 2008).

1.4 Ambient-temperature fimbriae(ATF)

Ambient-temperature fimbriae (ATF) were classified as a new fimbrial type, as examined by electron microscopy and immunogold labeling (Nielubowicz, 2010). The purified fimbria revealed a subunit with an apparent molecular size of 24 kDa, where the *N*-terminal amino acid sequence does not demonstrate similarity to that of any other fimbriae (Rocha et al., 2007). These fimbriae are produced in abundance at 25° C and are also synthesized to a lesser degree at 30 and 37° C. The genes responsible for the production of ATF are organized in the *atf* gene cluster and encode a 19-kDa major-subunit AtfA (Nielubowicz, 2010). Atf sequences represent a minimal fimbrial gene cluster. The atf gene cluster, composed of three genes, *atfA*, *atfB*, and *atfC*, appears to carry the oretical minimum number of genes necessary to encode atypical enteric fimbria. A structural subunit (*AtfA*), a chaperone (*AtfB*), and a molecular usher (*AtfC*) are encoded by contiguous genes. No putative regulatory or minor pilin genes are evident (Zunino *et al*., 2000).

1.5 Objective of research

The present study aims to isolate and identify *Proteus vulgaris* from urine samples and

detection of fimbrial genes (*mrp*, *mrkA*, *atf*, *uca*) in *P. vulgaris* isolates.

1.6 Justification of research

Proteus strains must attach to uroepithelial cells to colonize and initiate UTI and may express a variety of adhesins to assist in this initial attachment. These adhesins also contribute to the direct triggering of host and bacterial signaling pathways, assisting in the delivery of bacterial products to host tissues, and promoting bacterial invasion into host cells.

1.7 The purpose of this research

The object of this review is *Proteus vulgaris*, which considered now to belong to the opportunistic pathogens. Widely distributed in nature (in soil, water, and sewage), *P. vulgaris* play a significant ecological role. When present in the niches of higher macroorganisms, these species are able to evoke pathological events in different regions of the human body. The invaders *P. vulgaris* have numerous virulence factors including fimbriae, flagella, outer membrane proteins, lipopolysaccharide, capsule antigen, urease, immunoglobulin A proteases, hemolysins, amino acid deaminases, and swarming growth. So, if we detected these virulence factors we can tested or researched about factors that can be used to attenuate these factors and then the pathogenicity of this bacteria.

2. Expiremental

2.1 Bacterial isolates

Proteus vulgaris was isolated from a patient with UTI who were admitted to four hospitals: Babylon Hospital for Maternal and Pediatrics, Al-Hilla Surgical Teaching Hospital, Al-Hashymia hospital and Al-Qasim hospital during the period from 4/2012 to 1/2013.Standard biochemical tests were used for detecting *P.vulgaris* strains and by Vitek system (BioMerieux, USA) (Pincus, 1998; MacFFadin, 2000).

2.2 Mannose-resistant haemagglutination Assay (MRHA)

The haemagglutination was detected by clumping of erythrocytes by fimbriae of bacteria in the presence of D-mannose. This test was carried out as per the direct bacterial haemagglutination test - slide method and mannose-resistant haemagglutination tests (Old and Adegbola, 1985).The strains of *P. vulgaris* were inoculated into 1% nutrient broth and incubated at 37°C for 48 hours for full fimbriation. A panel of red blood cells was selected by obtaining blood from human (blood group 'O'). The red blood cells which were then washed thrice in normal saline and made up to a 3% suspension in fresh saline. They were used immediately or within a week when stored at 3-5° C. One drop of the RBC suspension was added to a drop of the broth culture and slide was rocked to and fro at room temperature for 5 minutes. Mannose-sensitive haemagglutination was detected by the absence of haemagglutination in a parallel set of test in which a drop of 2% w/v d-mannose was added to the red cells and a drop of broth culture. Mannose-resistant haemagglutination was detected by the presence of haemagglutination of 3% 'O' group human RBC in the presence of 2% mannose (Vagarali et al., 2008).Bacterial agglutination of tannic acid treated human erythrocytes (MR/K agglutination) was performed as previously described by Ong et al. (2010) to detect the expression of the expression of Type 3 fimbriae.

2.3 DNA Extraction and Genes Amplification

Genomic DNA was extracted by a commercial nucleic acid extraction kit (Bioneer-Korea) according to the manufacturer's instructions.

2.4 PCR Amplification of fimbrial genes

Amplification of fimbrial genes were performed in thermal cycler (MJ Reasearch, USA) using primers designed by this study using NCBI GenBank and MP Primer design online. The GenBank accession no.: (*mrp*:Z32686 (Bahrani and Mobley, 1994; Sosa et al., 2006), *uca*: U28420 (Cook et al., 1995; Sosa et al., 2006). *Atf*:z78535 (Massad *et al*., 1996; Zunino et al., 2000), *mrkA*: FJ96754, FJ96756-FJ96774, and FJ96777-FJ96789(Jeanmougin et al., 1998; Ong et al., 2010). These primers were provided by (Bioneer Company, Korea). Briefly each reaction was carried out in 25 µl reaction volume using 12.5 µl of Accustart™ Taq PCR Super Mix(VWR-USA), 1 µlof primers, 2 µl of DNA template, and 8.5 µl of Nuclease free water (ddH_2O).The primers and PCR conditions used to amplify genes encoding fimbriae with PCR are listed in table (1).

2.5 Detection of amplified products by agarose gel electrophoresis

Successful PCR amplification was confirmed by agarose gel electrophoresis, The PCR products were assessed by electrophoresis (Amercham Biosciences, USA) in 1% agarose gel with 0.5% ethidium bromide (Alfa Aesar, USA).

Table 1: Primers sequences and PCR conditions used to amplify fimbrial genes

Genes	Primer sequence (5'-3')	Size of product bp	PCR condition
mrp F *mrp* R	ATTTCAGGAAACAAAAGATG TTCTTACTGATAAGACATTG	565bp	94º C 3min 1x 94º C 30 s 40º C 30 s 30x 72º C 30 s 72º C 7min 1x
mrkA F *mrkA* R	AGAGTTTGATCMTGGCTCAG AAGGAGGTTATCCANCCRCA	416bp	94º C 3min 1x 94º C 30 S 45º C 30 S 30x 72º C 30 S 72º C 7min 1x
atf F *atf* R	CATAATTTCTAGACCTGCCCTAGCA CTGCTTGGATCCGTAATTTTTAACG	382bp	94º C 3min 1x 94º C 1min 50º C 1min 30x 72º C 1min 72º C 5min 1x
*uca*F *uca*R	AACCAGTTCCGCGTTGGCCTGG CGGAACGGCCTGACGTTGCAT	580bp	94º C 3min 1x 94ºC 30 s 50ºC 30 s 30x 72ºC 30 s in 72ºC 7min 1x

Agarose gel was prepared by dissolving 0.45 gm of agarose powder in 40ml of TBE buffer (pH:8) in Microwave(Kenmore, USA),allowed to cool to 50° C and then ethidium bromide at the concentration of 0.5 mg/ml was added (Sambrook and Rusell, 2001). The comb was fixed at one end of the tray for making wells used for loading DNA sample. The agarose was poured gently into the tray, and allowed to solidify at room temperature for 30 min. The comb was then removed gently from the tray. The tray was fixed in an electrophoresis chamber filled with TBE buffer that covered the surface of the gel, 5 µl of DNA sample mixed with Gel Loading Dye Blue (Biolabs, USA) was transferred intothe wells in agarose gel, and in one well we put 1 µl of DNA ladder (Biolabs, USA).The electric current was allowed at 110 volt for 60min. UV Trans-illuminater (San. Gabriel, USA) was used for the observation of DNA bands, and then gel was photographed using a Gel Documentation System with a digital camera (Bio Rad, USA).

2.6. Statistical methods

Data were statistically described in terms of frequencies (percentages). All statistical calculations were done using Microsoft Excel 2007(Microsoft Corporation, New York, USA).

3. Results

In order to detect *mrp*, *mrkA*, *uca and atf* fimbrial genes in *P. vulgaris* isolates, PCR was performed using designed specific primers and genomic DNA from each *P. vulgaris* isolate. PCR amplification of fimbrial genes indicated that all isolates carried those fimbrial genes. In all cases, *P. vulgaris mrp*, *mrkA*, *uca* and *atf* fimbrial genes amplified by PCR exhibited the predicted sizes (565, 416, 580, and 382bp respectively) .These results show in figure (1), (2), (3) and (4) respectively.

P. vulgaris strains were examined for MR/P and MR/Kfimbriae genotypically and also phenotypically by using haemagglutination assay (HA).The results of haemagglutination assay showed all isolates (100%) agglutinate human red blood cells in the presence of mannose and that confirmed by showing all *P. vulgaris* isolates have *MRP* and *MRK genes* (Figure1).

Other than MR/P fimbriae are known to lesser extent MR/K fimbriae. MR/K hemagglutinins were found more frequently on *P. penneri* strains than on *P. vulgaris and P. mirabilis* strains and most probably they facilitated the adherence of bacteria to the urinary catheters (Jacobsen *et al*., 2008). Whereas, in the present study, it has been detected the

Figure 1: Gel electrophoresis of PCR of *mrp* amplicon product. M: Marker, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10: no. of *P. vulgaris* isolates, *mrp* amplicon size: 565bp

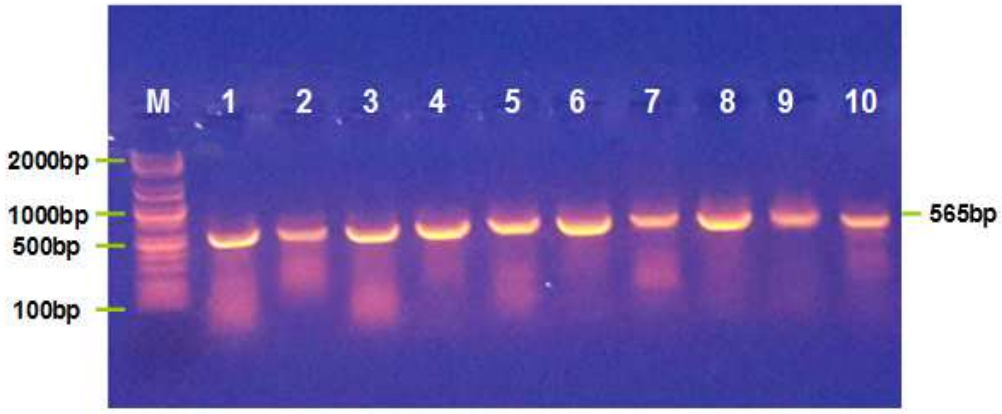


Figure 2: Gel electrophoresis of PCR of *mrkA* amplicon product. M: Marker, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10: no. of *P. vulgaris* isolates, *mrkA* gene amplicon size: 416 bp

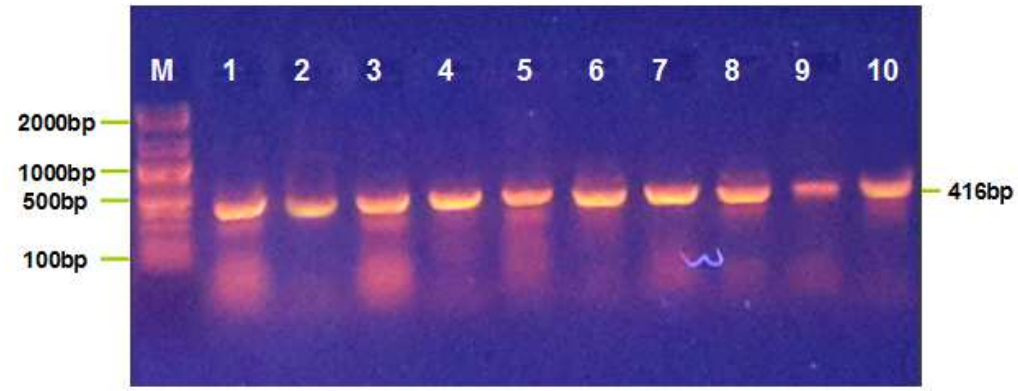
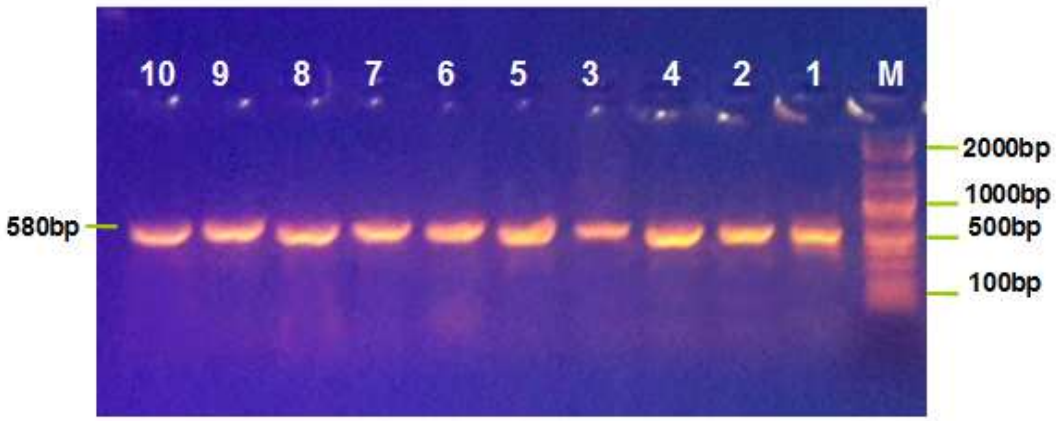


Figure 3: Gel electrophoresis of PCR of *uca* amplicon product. M: Marker, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10: no. of *P. vulgaris* isolates, *uca* gene amplicon size: 580bp

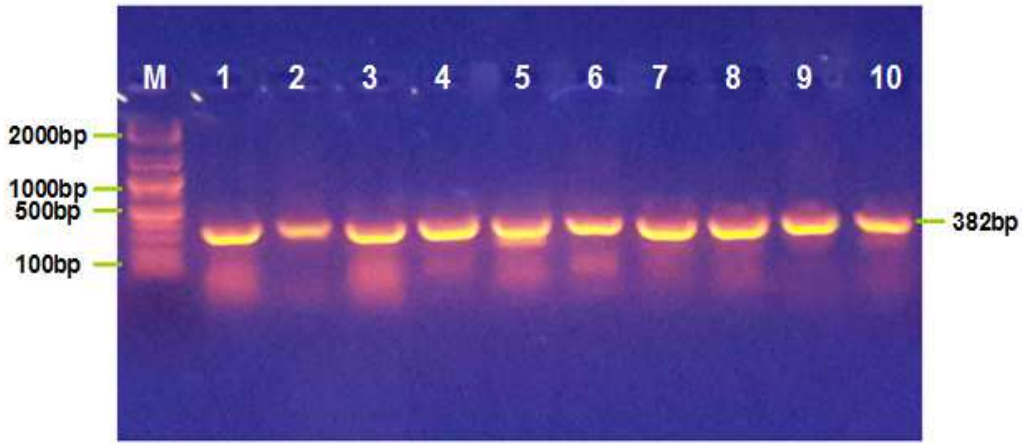


presence of this type of fimbrial gene (*mrkA*) in all *P. vulgaris* isolates; the results show in figure (2).

Besides MR/P and MR/K fimbriae, the other fimbriae produced by *Proteus during* UTIs are surface adhesins; UCA/NAF and ATF fimbriae which determined not to be involved in the hemagglutination caused by MR/P and MR/K fimbriae (Jacobsen *et al*., 2008). A small collection of *P. vulgaris* strains revealed that all isolates produced *uca* (Figure 3).

P. vulgaris isolates have been screened for production of ATF and all strains tested were positive (Figure 4).

Figure 4: Gel electrophoresis of PCR of *atf* amplicon product. M: Marker, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10: no. of P. *vulgaris* isolates, *atf* gene amplicon size: 382bp



4. Discussion

Adhesion mediated by fimbriae is a crucial step for successful bacterial colonization of the urinary tract mucosa and has an important role in the pathogenesis of UTI. *Proteus*, a common uropathogen, can express several types of fimbriae simultaneously, including MR/P, MR/K, UCA and ATF fimbriae (Coker et al., 2000; Zunino et al., 2007).

Historically, fimbriae of *Proteus spp.* were characterized by their hemagglutination (HA) properties and fell into two classes based on this activity: mannose-resistant/*Proteus*-like (MR/P) and mannose resistant / *Klebsiella*-like (MR/K). As the names imply, HA activity of both types of fimbriae was resistant to mannose (*i.e.*, addition of mannose did not inhibit HA); fimbriae with MR/P activity resulted in agglutination of fresh (but not tannin-treated) erythrocytes, while fimbriae with MR/K activity resulted in agglutination of tannic acid-treated erythrocytes (Nielubowicz, 2010; Rozalsk et al., 2012). The results of haemagglutination assay showed all *P. vulgaris* isolates agglutinate human red blood cells in the presence of mannose. These results were similar to that results reported by Mishara et al. (2001) who found (91) out of (148) (61.49%) *Proteus* isolates gave positive result of CFA in the presence of D- mannose. Also, they were in agreement with results of Sosa*et al*. (2006), who observed that every tested isolate of *Proteus* spp. was able to agglutinate fresh human erythrocyte and this reaction was not inhibited by D-mannose.

Initial studies revealed that sera from infected mice reacted strongly to MR/P fimbrial preparations, which indicates these fimbriae are expressed *in vivo* (Nielubowicz, 2010). The expression of MR/P fimbriae appears to be highly induced during infection (Li *et al*., 2002).Culture under oxygen limitation *in vitro* induces MR/P expression (Lane *et al*., 2009). Since the urinary tract is proposed to be oxygen-limited (Snyder *et al*., 2005), this condition could contribute to the upregulation of MR/P fimbriae observed *in vivo* (Nielubowicz, 2010). Other studies have suggested that MR/P fimbriae play a role in the virulence observed during UTIs caused by uropathogenic *P.mirabilis* strains. In the mouse CBA model of ascending UTI, infection with *P. mirabilis* elicited a strong immune response to *mrpA*, the major structural subunit of MR/P fimbria, indicating that MR/P fimbriae were expressed *in vivo* (Jacobsen *et al*., 2008). Isogenic mutants incapable of expressing MR/P fimbriae were attenuated when examined in this mouse model (Li *et al.*, 1999*;* Li *et al.*, 2002). A mutant constitutively expressing MR/P fimbriae outcompeted the wild-type strain in the murine bladder but not the kidneys in a co-challenge experiment, thereby establishing MR/P fimbriae as being an important bladder colonization factor for *Proteus spp.* (Li et al., 2002). Tissue binding studies by Sareneva et al. (1990) revealed the propensity of this fimbrial type to adhere specifically to the human renal tubular epithelial cells and to the exfoliated uroepithelial cells of urinary sediment.

MR/K hemagglutinins were detected in all *P. vulgaris* isolates (Fig. 2). Since hemagglutinin was originally characterized in *Klebsiella* strains, the fimbrial adhesin has been referred to as the mannose-resistant, *Klebsiella*-like (MR/K) hemagglutinin (Ong et al., 2008). Our results agree with other results obtained by

Bijlsma et al. (1995) and Cook et al. (1995) who found that the 540bp *ucaA* gene that encodes the major fimbrial subunit of UCA has nucleic acid homology (58%) to the f17a gene of *E. coli* F17 pilin and was identified in all 26 *P. mirabilis* strains tested. Several studies have clearly demonstrated that type 3 fimbriae also mediates various adherence functions such as binding to epithelial cells and extra-cellular matrix proteins for instance, collagen V (Ong et al., 2008 and Sonbol et al., 2012). Several observations support a role for one of these hemagglutinins, MR/K (type3) fimbriae, in the virulence of uropathogenic *P.mirabilis* within the urinary tract. This species has been shown to adhere *in vitro* to human uroepithelial cells (Bahrani et al., 1993 and Rocha et al., 2007). A strain expressing the MR/K hemagglutinin was shown to bind specifically to components of sectioned human kidney, namely, Bowman's capsule of the glomeruli and tubular basement membrane (Sareneva et al., 1990 and Ong et al., 2010).Unlike the adherence mediated by the MR/P hemagglutinin, binding to kidney tissue by MR/K-expressing strains was not inhibited by low-molecular-weight components present in urine and thus was suggested to be more likely to play a role *in vivo* than MR/P fimbriae. In addition, Silverblatt (1974) demonstrated that fimbriae morphologically consistent with MR/K fimbriae were expressed in the kidney of an experimentally infected rat. MR/K of other species (i.e. *Klebsiella* and *Providencia* spp.) have also been implicated in adherence to tissues and inert surfaces (Bahrani et al., 1993). While these observations support a role of MR/K fimbriae in pathogenesis they by no means constitute proof that this adhesion contributes to virulence where there are, also suggestions that MR/K fimbriae do not play a role in pathogenesis. Silverblatt and Ofek (1978) demonstrated in a rat model of pyelonephritis that MR/K fimbriae were expressed *in vivo* but that, overtime, the Bacterial population shifted away from production of this fimbrial type towards the production of MR/P fimbriae. Animals inoculated with bacteria predominantly producing MR/P fimbriae developed more severe lesions in the kidney than animals inoculated with the bacterial strain cultured in such a fashion so that MR/K fimbriae predominated (Bahrani et al., 1993 and O'May et al., 2008).A putative regulatory gene (*mrk E*) located upstream of *mrkA* has been described previously in *K. pneumoniae*. These genes have been shown to reside in multiple genomic locations, including the chromosome, conjugative plasmids, and within a composite transposon. Transfer of a*mrk*-containing conjugative plasmid to strains of *Salmonella entricaserova typhimurium*, *K. pneumoniae*, and *E. aerogenes* species has also been demonstrated (Sonbol et al., 2012). Taken together, these data strongly support the spread of the *mrk* genes between Gram-negative pathogens by lateral gene transfer.

Also in this study all *P. vulgaris* strains were showed to have *uca* gene (Fig. 3). Wray *et al*. (1986) characterized UCA, a NAF from *P. mirabilis* HU1069 that was demonstrated to weakly attach to exfoliated human desquamated uroepithelial cells. These fimbrial subunits were isolated and characterized from a *P. mirabilis* strain 7,570 isolated from a patient with struvite urolithiasis and renamed nonagglutinating fimbriae (NAF) by Tolson et al. (1995).Bacteria expressing NAF adhered strongly to a number of cell lines *in vitro*, including uroepithelial cell, MDCK (Madin-Darby canine kidney, and EJ/28 urinary tract tumor cell lines (Latta *et al*., 1998). Because of its homology to f17a gene of *E. coli* F17 fimbriae that assist in intestinal tract colonization, it is possible that these fimbriae play a role in the initiation of UTIs by allowing *Proteus* to attach and establish in the intestines and thus form a reservoir of organisms that can potentially cause UTIs. However, there have been no definitive studies examining this possibility (Rozalsk et al., 2012).

Other fimbriae gene have been detected in *P. vulgaris* isolates was ambient temperature fimbriae (ATF) .They named ATF because they are expressed optimally during static culture in Luria broth at 23° C. They are also expressed, to a lesser degree, during static and aerated culture in Luria broth at 37° C, but not during culture in minimal medium, on agar plates, or at higher temperatures (such as 42° C) (Nielubowicz, 2010). Expression of ATF does not correlate with hemagglutination (Zunino et al., 2000).This finding corresponding with other study about *P. mirabilis* ATF fimbriae by Massad et al. (1994) and Rocha et al. (2007) who found all examined strains of *P. mirabilis* expressed ATF to varying degrees and as for other enteric fimbriae, the expression of ATF was affected by environmental conditions.

Zunino et al. (2000) isolated a mutant of *P. mirabilis* that was unable to synthesize ATF. They confirmed that ATF are not required for *P. mirabilis* hemagglutination. This ATF mutant was also used in a mouse ascending UTI

model and no significant differences were noted between numbers of infected animals or organs, or between the levels of colonization of kidney or bladders between the wild-type or mutant infections. This indicates that ATF mutagenesis did not affect the infective ability of *Proteus*. Therefore, ATF are more likely to play a role in the survival of *Proteus* in the external environments of a mammalian host due to its optimal expression temperature. So, ATF fimbriae are not important in the pathogenicity of human beings. Whether ATF play a role in colonization of the urinary tract is difficult to assess. Thus far, no receptor-binding function can be identified. Bacteria expressing ATF do not agglutinate any erythrocyte species. Furthermore, these fimbriae are optimally expressed at 23° C and are less prominent at 37° C. While this suggests that ATF may not contribute significantly to colonization of the mammalian host, we cannot rule out the possibility that these fimbriae are expressed at high levels *in vivo*. For example, cholera toxin is optimally expressed by *Vibrio cholera* in vitro at temperatures lower than 37° C, although the toxin is produced during infection (Massad et al., 1994).

Conclusion

The study has arrived at the following conclusions; *Proteus vulgaris* was predominant among patients with UTI and all *P. vulgaris* isolates in this study have the ability to possess more than one adhesive factor genes such as (*mrp*, *mrkA*, *uca* and *atf*).

Limitations

This research is limited potential to be leveraged only on detection of fimbrial genes in *Proteus vulgaris* isolates but we need further facilities as genetic analyzer system to do sequencing for these detected genes from *P. vulgaris*.

Recommendations

There are still many things that are unknown concerning *P. vulgaris* adhesive factors and virulence factor as the all including the method by which these organisms produce these structures. So, we recommended to do further study about these virulence factors, especially adhesive factors which would lead to a better understand of the general process of fimbrial development during *Proteus* infection and could indicate potential targets for prevention and treatment of these types of infections.

Funding and Policy Aspects

We recommended Funding and Policy Aspects to provision possibilities and material support from developmental systems and the source of funding to support the researchers to go on for discovering and investigating the scientific facts that can be support the general economy and reduce material loses.

Authors' Contribution and Competing Interests

We are so contributing in this research and there are no competing interests.

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