

EFFECT OF BLUEBERRY EXTRACT ON PATHOGENIC STRAINS *ESCHERICHIA COLI* AND *PROTEUS MIRABILIS*

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ABSTRACT. The most frequently encountered uropathogens are aerobic and facultative anaerobic Gram–negative bacteria mainly *Escherichia coli*. *Proteus mirabilis* is also an opportunistic Gram-negative uropathogen that infects the upper urinary tract. Coliforms and *Proteus* spp. currently cause 29% of nosocomial infections. In the present study was examined the effect of blueberry extract on some virulent properties of four strains *Escherichia coli* and six strains *Proteus mirabilis* which had been isolated from urinary tract infections. Bacterial strains were cultivated in LB medium with blueberry extract. The study of motility after cultivation showed that almost all strains became nonmotile. Blueberry extract caused full loss of hemagglutination abilities of bacterial strains. In addition it was found that *Escherichia coli* and *Proteus mirabilis* showed decreased serum resistance after cultivation in the presence of blueberry extract. The results suggest that blueberry extract have a potential in the treatment and prevention of urinary tract infections.

KEY WORDS. APUD cells, endocrine cells, human embryo, gastrointestinal tract.

INTRODUCTION

Humans currently among the most common bacterial infections acquire urinary tract infections (UTIs). The bladder is the primary site of infection in about 95% of all UTIs. Infection of the kidney (pyelonephritis) is usually a more serious problem and is associated with flank pain, nausea, vomiting, fever, sweats, malaise, and potentially the symptoms that are characteristic of cystitis. In about 30% of cases pyelonephritis is complicated by bacteraemia which may lead to sepsis. Most uropathogens originate in the intestinal flora and enter the bladder by an ascending route, via the urethra with an interim phase of periurethral and distal urethral colonization (SOBEL, 1997).

The primary causative agents of UTIs, accounting for greater than 80% of these infections, are strains of uropathogenic *Escherichia coli* (UPEC) (HOOTON,

STAMM, 1997; SVANBORG, GODALY, 1997). UPEC isolates, like enteric *E. coli* pathogens, are a genetically heterogeneous group and can vary significantly in their abilities to colonize and persist within either the bladder or the kidneys (JOHNSON *et al.*, 1998; ZHANG *et al.*, 2000). Epidemiological, cell culture and animal studies have indicated that a number of factors encoded by UPEC can modulate bacterial virulence within the urinary tract. These virulence factors are usually encoded on the chromosome of UPEC and are often part of large, unstable chromosomal regions known as pathogenicity islands (HACKER, KAPER, 2000; MUHLDORFER *et al.*, 2001). Virulence factors associated with UPEC include the toxins α -haemolysin and cytotoxic necrotizing factor-1 (CNF-1), the siderophores aerobactin and enterobactin, lipopolysaccharide (LPS), capsules, and a number of adhesive organelles (JOHNSON, 1991; MUHLDORFER *et al.*, 2001). The presentation of adhesive molecules (adhesins) by UPEC, more so than the expression of toxins or other virulence factors, is the most important determinant of pathogenicity. Adhesins enable UPEC to bind to host cells within the urinary tract and avoid rapid clearance with the bulk flow of urine. Adhesins can also contribute to virulence in a number of other ways; directly triggering host and bacterial cell signalling pathways, facilitating the delivery of other bacterial products to host tissues, and promoting bacterial invasion (MULVEY, 2002).

Proteus mirabilis, a common uropathogen, causes urinary tract infections (UTIs) in individuals with structural abnormalities of the urinary tract and is frequently isolated from the urine of elderly patients undergoing long-term catheterization. Complications from infection with this organism are frequently serious and include bladder and kidney stone formation, encrustation and obstruction of the urinary catheter, acute pyelonephritis, and bacteremia. Indeed, this bacterium has a propensity for colonization of the kidney; in bladder washout studies, *P. mirabilis* was found in the kidneys more often than *Escherichia coli* (JANSEN *et al.*, 2003). Several virulence factors have been identified and characterized for *P. mirabilis*. These factors include a potent urease that catalyzes formation of ammonia from urea and leads to urinary stone formation, a pore-forming hemolysin, ZapA metalloprotease which cleaves both immunoglobulin G (IgG) and IgA, a capsular polysaccharide, four distinct fimbrial types, and peritrichous flagella for swimming and swarming motility (ALLISON *et al.*, 1992; ALLISON *et al.*, 1994; MOBLAY, BELAS, 1995; LI *et al.*, 1999; FRASER *et al.*, 2002). It is believed that the ability of *P. mirabilis* to colonize the surfaces of catheters and the urinary tract may be aided by the characteristic first described over a century ago and presently referred to as swarmer cell differentiation and behavior (BELAS *et al.*, 1998).

Traditionally UTIs are treated with antibacterial drugs, but these are expensive, can have side effects, and may lead to resistance. Research is showing that blueberries contain a number of compounds that have medicinally beneficial properties. The earliest recorded use of blueberry for medicinal purpose dates from the Middle Ages, and it has been used in European folk medicine since the 16th century. Some of its reported medicinal benefits include preventing urinary tract infections, antioxidant (anti-cancer) activity, reducing heart disease risk,

strengthening collagen, regulating blood sugar, improving night vision, reducing replication of the HIV virus, and treating diarrhea.

This study examined the effect of blueberry water extract on some virulence properties of uropathogenic strains *E.coli* and *P.mirabilis*. The strains are urine isolates from patients with chronic UTIs.

MATERIAL AND METHODS

1. Bacterial strains and culture.

The strains used in the study were isolated from urine of patients with chronic UTIs. Urine isolates were obtained in the clinical microbiology laboratory at State's hospital of Plovdiv. *Escherichia coli* and *Proteus mirabilis* were grown in LB broth or on LB agar plates at 37°C.

2. Hemagglutination (HA) assays.

Venous blood from donors (blood type ORh+ and ARh+) was collected with 10% sodium citrate in 1:1 ratio. The mixture was centrifuged at 2500 rpm for 10min. The erythrocytes were washed 5x with 0.07 M PBS (pH 7.2). The washed erythrocytes were suspended in PBS to 3% and kept for week at 4°C. HA assays were performed as previously described (KALLENIOUS G. et. al., 1980) in presence or absence of D-mannose at 4°C for 1 h and 25°C for 10 min.

3. Serum resistance.

Resistance of the strains to normal serum was tested as described earlier (STOITSOVA et. al., 2004) using 80% human serum from healthy donors. The bacteria were incubated in the serum for 1, 2 or 3 hours and then plated, after serial dilutions, on nutrient agar. The number of colony forming units was counted after an overnight growth at 37°C, and was estimated as % of the control. Strains were defined as 'sensitive' where less than 1% of cells survived, as 'resistant' where 90% and more of the cells survived, and the other results were considered as 'intermediary'.

4. Effect of blueberry water extract (BWE) on *E.coli* and *P.mirabilis* strains.

Extract was prepared from 50g dry blueberry boiled for 5 min in 500ml dH₂O. After 30 min the blueberry extract was filter sterilized and added 1:1 to LB broth. After cultivation overnight the hemagglutination abilities, serum resistance and motility of strains were estimated.

RESULTS AND DISCUSSION

Bacterial adherence to mucosal surfaces, a prerequisite for the development of most mammalian infections, is facilitated by fimbriae, which are proteinaceous fibers on the bacterial wall. Fimbriae produce adhesins that attach to specific monosaccharide or oligosaccharide receptors on uroepithelial cells. The mannose-resistant adhesins of *E.coli* strains exhibiting mannose-resistant hemagglutination (MRHA) are diverse. On the basis of receptor specificity, these adhesins can be considered two groups (JOHNSON, 1991). Bacterial strains, tested in present study, showed different abilities for hemagglutination of blood type O and A

erythrocytes in presence or absence of D–mannose (tabl.1). These hemagglutination patterns suggest presence of different type adhesins (type 1, P pili and others).

Table 1. Hemagglutination of *E.coli* and *P.mirabilis* strains of human erythrocytes (blood type ORh⁺ and ARh⁺)

Штам №	Hemagglutination with type ORh ⁺				Hemagglutination with type ARh ⁺			
	25°C		4°C		25°C		4°C	
	– M	+M	– M	+M	– M	+M	– M	+M
<i>E.coli</i> 3A	+	+	+	+	–	+	–	–
<i>E.coli</i> 14A	+++	+++	+++	+++	+	+	–	–
<i>E.coli</i> 15A	++	++	++	–	+	++	–	+
<i>E.coli</i> 26A	+	–	–	–	+++	+	+	–
<i>P.mirabilis</i> 10A	–	–	–	–	–	+	–	–
<i>P.mirabilis</i> 18A	–	+	–	–	+	++	–	+
<i>P.mirabilis</i> 19A	–	+	–	–	++	++	–	+
<i>P.mirabilis</i> 20A	–	+	–	–	–	++	–	–
<i>P.mirabilis</i> 21A	–	–	–	–	++	+++	++	+
<i>P.mirabilis</i> 24A	–	+	–	–	+	–	+	+

" +, ++, +++ " – strength of hemagglutination
 "M+" – hemagglutination in presence of 1% D–mannose
 "M–" – hemagglutination without 1% D–mannose

Proteus mirabilis, commonly associated with complicated urinary tract infections (UTIs), expresses several types of fimbrial structures that promote attachment to and colonization of host mucosal surfaces. One of these, mannose–resistant *Proteus*–like (MR/P) fimbria, a surface structure responsible for mannose–resistant hemagglutination (MRHA), has been shown to contribute significantly to the development of experimental UTIs. The majority of *P. mirabilis* strains isolated from patients with acute pyelonephritis express MR/P fimbriae as a single hemagglutinin type. Recent studies on the expression of MR/P fimbriae at the transcription level show that the element which regulates transcription in a manner similar to *Escherichia coli* type 1 fimbria is >98% turned on in vivo (in the urine, bladder, and kidneys of infected mice) versus at most 50% in vitro (static culture) (LI et.al., 1999).

After cultivation in presence of BWE the studied *E.coli* and *P.mirabilis* strains lose all abilities for MSHA and MRHA at 4°C and 25°C.

KOŁODZEJ (1999) showed antimicrobial effect of 27 tannins against *E.coli*, *Proteus mirabilis* and *Pseudomonas spp.* Proanthocyanidins in blueberry can reduce nearly at 100% expression of P fimbriae. Inhibition was supported with elongation of bacterial cells. Fructose inhibits adhesion of strains possessing type 1 fimbria (OFEK et.al., 1991). Epicatechin is a bioflavonoid found both in cranberries and blueberries. It is believed to inhibit the attachment of the bacteria to the lining of the bladder thus

causing the bacteria to be eliminated in the urine rather than attaching to the bladder wall, multiplying and causing an infection. Some components of blueberry extract cause decrease of pH of urine (OFEK et.al., 1991).

Cultivation of strains in presence of blueberry extract leads to reduction of motility as shown in tab.2. Inhibition of motility of strains *E.coli* and *P.mirabilis* is probable result of formation of complex between flagelin and tannins in blueberry extract.

Table 2. Effect of blueberry extract on motility of uropathogenic strains *E.coli* and *P.mirabilis*

Motility	Escherihia coli				Proteus mirabilis					
	3A	14A	15A	26A	10A	18A	19A	20A	21A	24A
Growth in LB	+	+	+	+	+	+	+	+	+	-
Growth in LB + blueberry extract	-	-	-	-	-	+	+	-	-	-

Proteus mirabilis 18A and 19A retain their motility. During *Proteus mirabilis* differentiation into elongated hyperflagellated swarm cells, *flhDC* transcription is strongly but transiently increased (CLARET L., HUGHES C., 2000).

Serum resistance is one of potential virulent factors of uropathogenic bacteria. Clinically isolated strains of *E.coli* and *P.mirabilis* showed different sensitivity to normal serum in the present study. After cultivation in presence of BE all strains displayed significant reduction in their resistance to normal human serum. This increased sensitivity may be an effect of inhibition of adhesins involving in blocking of complement activation.

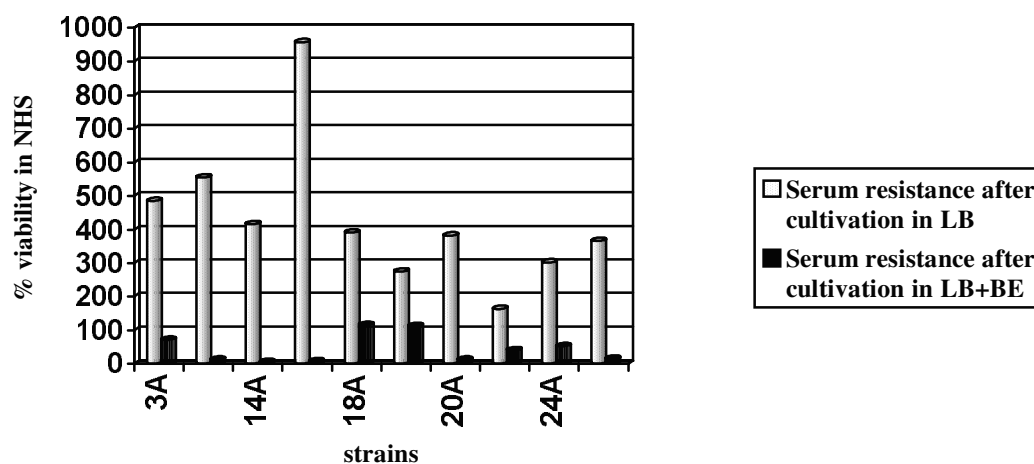


Fig.1. Effect of cultivation in presence of blueberry extract (BE) on serum resistance of uropathogenic strains *E.coli* and *P.mirabilis*

The use of cranberry among individuals to prevent or treat UTI is a common practice. The accumulating evidence from small, non-controlled and controlled

clinical trials suggests that berries may relieve symptoms associated with UTI and may reduce the need for antibiotics. The components of blueberries inhibit the binding of *E. coli* bacteria to the urinary tract wall, thus preventing the bacteria from invading the tissues and causing an infection. Recent work indicates that blueberry water extract contains compounds having anti-adhesion and immunomodulatory properties. It is possible to use dry blueberry when is not the season for them in treatment and prophylaxis of UTI.

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