

The Study of Uropathogenicity Factors for Escherichia Coli Strains

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ABSTRACT: Urinary tract infection (UTI) is the most common form of extraintestinal Escherichia Coli infection (E.coli), and E. coli is the most common cause of UTI. The aim of this paper is to study the uropathogenicity factors for some strains of E.coli involved in the etiology of UTI and the affiliation of urinary E.coli strains to the serogroups involved in the UTI. We studied 208 strains of E. coli from urine samples sterilely collected from patients with clinical suspicion of urinary tract infection. The study was conducted in Emergency County Hospital Craiova between 2012-2014. Out of the 208 strains of E. coli submitted to the study, 60 strains (28.84%) - MRHA with human red cells, 28 strains (13.50%) - MRHA human red cells and blood red cells MSHA with guinea pigs, and 44 strains (21.12%) - MSHA with guinea pig red blood cells; 76 strains (36.54%) - no hemagglutination. Regarding our study, 42,34% of E.coli strains presented human MRHA putting forward their potential to cause pyelonephritis. The 68 hemolytic strains (37,20%) of urinary E. coli were tested for the production of the cytotoxin, thus obtaining characteristic cytotoxic effect for 26 strains (38.20%) whereas its absence was registered in 42 strains (61.80%). E. coli O6 strains isolated from hospitalized adults are more frequently hemolytic than those isolated from the other groups and MRHA was more common in hemolytic strains of the same group O6). Mannose-resistant hemagglutination is more frequent in strains that develop Hly but do not produce CNF (Cytotoxic Necrotizing Factor), than in strains producing CNF.

KEYWORDS: *Escherichia coli, urinary tract infection, uropathogenicity factors*

Introduction

Urinary tract infection (UTI) is the most common form of extraintestinal Escherichia coli (E. coli) infection, and E. coli is the most common cause of UTI. E. coli are a very diverse species of bacteria found naturally in the intestinal tract of all humans and many other animal species. A subset of E. coli are capable of causing enteric/diarrhoeal disease, and a different subset cause extra-intestinal disease, including urinary tract infection (UTI). [1],[2]

A UTI is defined as a significant amount of pathogenic microorganisms in the urinary apparatus. Should symptoms such as painful, frequent urination or blood in urine be present, it is advisable to consider significant in terms of etiology about 100 uropathogenic bacteria per urine milliliter. However, many individuals experience no symptoms, a condition we describe as asymptomatic bacteriuria. On the contrary, cases involving symptomatic bacteriuria are classified either as cystitis when the infection is limited to bladder or pyelonephritis when the kidney is also

compromised. While cystitis in the otherwise healthy individual generally resolves without sequelae, pyelonephritis can cause serious morbidity and be fatal. Patients with abnormal or obstructed urinary tracts or dealing with compromised immune systems experience a seriously high risk of UTI. These infections are often referred to as complicated UTIs. There is an increased risk in this group that a simple urinary tract infection may progress to systemic infection. [3]

The relationship between the presence of bacteriological virulence factors and the severity of urinary tract infection (UTI) was the main point of interest brought forward in this study. The production of alpha-hemolysin (Hly), the expression of P-fimbriae and the mannose-resistant hemagglutination (MRHA) type IVa (associated with the presence of P-fimbriae), were factors detected more frequently in Escherichia coli strains from acute pyelonephritis than in strains isolated from cystitis and asymptomatic bacteriuria. In contrast, the production of type 1 cytotoxic necrotizing factor (CNF1) and the expression of

MRHA types III and IVb were distributed uniformly in strains causing different clinical categories of UTI. [4]

The adherence of E.coli strains to urinary epithelium cells is conferred by fimbrial and non-fimbrial adhesins, representing the most important factor in the pathogenesis of urinary tract infection. Fimbrial adhesins can be revealed "in vitro" by means of hemagglutination thus allowing the illustration of two types of urinary E. coli, one causing pyelonephritis and giving mannose resistant hemagglutination (MRHA) with human blood red cells, and the other responsible for lower urinary infections and inducing mannose-sensitive hemagglutination (MSHA) of the guinea pig erythrocytes. The presence of cytotoxic necrotizing factor (CNF) as a pathogenic factor in urinary infections has been demonstrated by A. Kaprioli and collaborators who have described the existence of two types of toxins called CNF1 and CNF2 which are linked to cell development and multinucleation on HeLa and Vero cells, rabbit dermonecrosis and death in mice. [5] .

Another pathogenic factor, the bacteriological alpha-hemolysin can contribute to the renal tubular cell damage for it increases the virulence of the bacteria by ensuring the lysis of erythrocytes and therefore provides the amount of iron necessary to growth. At the same time , it can also protect. [6], [7]

E.coli due to its ability to neutralize phagocytes. The genes encoding the hemolysin and CNF1 are closely related to the bacteriological chromosome of the urinary E.coli, while CNF2 has determinants located on the plasmids transmissible from animal strains. CNF1 induces a profound reorganization of the actin cytoskeleton and its polymerization makes possible the penetration of the originally non-invasive bacteria in Hep-2 cell line , highly suggesting that production of CNF 1 by uropathogenic strains can facilitate the passage of the bacteria from the urinary tract to the blood stream. [8]

Aim

The aim of this paper is to study the uropathogenicity factors of some strains of E.coli involved in the etiology of UTI and the affiliation of urinary E.coli strains to the serogroups involved in the urinary tract infections.

Materials and Methods

We studied 208 strains of E. coli from urine samples sterilely collected from patients with clinical suspicion of urinary tract infection, 38 isolated from children hospitalized in Pediatrics, 126 samples from adults hospitalized in urology and 44 samples from ambulatory children. The study was conducted in the Laboratory of Medical Analysis of Emergency County Hospital Craiova in between 2012-2014.

The following uropathogenicity factors present in E.coli strains were submitted to study:

- the presence of mannose resistant adhesins;
- the production of hemolysis;
- the production of cytotoxins (Cytotoxic Necrotizing Factor).

The urine sample was made in the morning during the first spontaneous urination, from the "middle stream", in strict compliance with the harvesting instructions and from the catheter in case of catheterized patients. We insisted that the urine collection to be made prior to antibiotics treatment. Urine samples were subjected to the following laboratory tests:

macroscopic examination - aiming to color and transparency;

bacteriostatic examination

The identification of isolated strains was based on cultural and morphological characters, as well as on the biochemical features.

Hemagglutination :

It was performed by means of agglutination on a slide of the bacteriological culture with A group human blood and guinea pig blood. The Bacteriological strains have been cultivated on ACF (antigen colonization factor) agar. Hemagglutination emergence was monitored during one minute and its intensity was appreciated with +, ++, +++ and +++++. The hemagglutination was noted as resistant (MRHA) ,which occurred in the presence of mannose and as sensitive (MSHA) which was absent or greatly reduced in the presence of mannose.

The emphasis of cytotoxicity was performed on Vero cell cultures; the cell line was maintained in Eagle's medium with 5% fetal calf serum and antibiotics (penicillin 100 IU / ml and streptomycin 100 / ml). The cytotoxin effect was manifested by the almost complete destruction of the cell layer, belonging to the microscopic field to scattered retatinated or partially damaged cells with characteristic morphology.

Hemolysin highlighting:

The hemolysin highlighting was made by growing the tested strains in agar-5% sheep

blood and incubating them for 24 hours at 37°C.

Serological Identification:

We performed agglutination reactions on the slide using anti *E. coli* O1 - D25 immune sera, in order to establish the affiliation of the strains to a serogroup involved in the etiology of urinary infections and immune monovalent and polyvalent anti enteropathogenic *E. coli* sera. The positive reaction is translated by the emergence of clusters thickening, fluid classification. For a correct interpretation of the reaction we watched the witness made from a

drop of normal saline solution and a loop of bacteriological culture which must have lactescent aspects.

Results and Discussions

Out of the 208 strains of *E. coli* submitted to the study, 60 strains (28.84%) showed MRHA with human red cells, 28 strains (13.50%) had both MRHA human red cells and blood red cells MSHA with guinea pigs, and 44 strains (21.12%) experienced MSHA with guinea pig red blood cells; 76 strains (36.54%) revealed no hemagglutination (Fig.1).

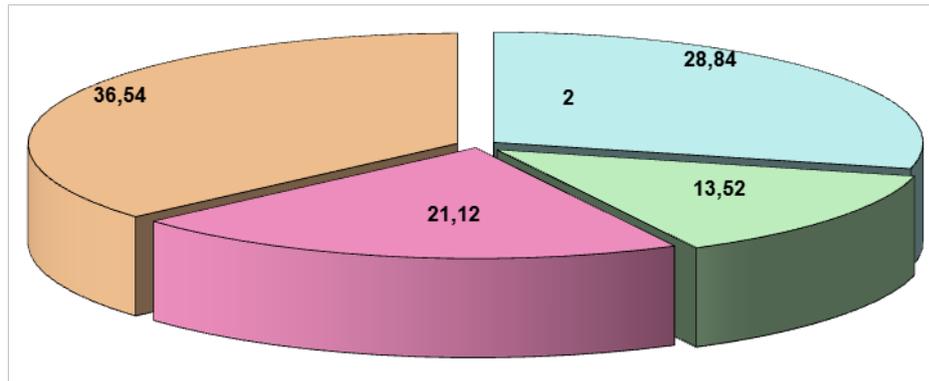


Fig.1. Mannose resistant hemagglutination (MRHA) and mannose sensitive hemagglutination (SMHA) of urinary *E. coli* strains

The literature correlates human MRHA with the adherence of *E. coli* strains to different areas of the renal parenchyma tissue, therefore constituting the evolutionary stages of the pathogenic mechanism in pyelonephritis. Regarding our study, 42.34% of *E. coli* strains presented human MRHA putting forward their potential to cause pyelonephritis. We encountered similar results in the papers of Blanco and collaborators who discovered human MRHA in 23-42% of the urinary *E. coli* strains submitted to study. [9]

Type 1 fimbriae associated with mice MSHA have a role in the urothelial colonization, interfering in the pathogenesis of pyelonephritis only if the bacteria from a low-end urinary tract infection reaches the kidney through a vesico-urethral reflux. We found this type of fimbriae in 21.12% of the strains studied alone and in 13.50% of the strains associated with MRHA. We discovered references about the coexistence of these two types of fimbriae in specialty literature, but without any special remark on uropathogenicity.

The hemolysin production and the correlation with urinary MRHA *E. coli* strains

The presence of Hly was highlighted in 68 strains (32.70%), similar to the values between 38-51% (8.25) obtained by other authors. During the experiment we observed that the studied strains are highly likely to lose their hemolysis capacity. Hereby out of the 52 strains retested in order to highlight this feature, after a three month time lapse, only 28 strains still presented this capacity. Hemolysin production is determined not only by chromosomes but also by plasmids. Orskov and collaborators commonly got this toxin production more frequently from strains isolated from high urinary infections (51%) than from those isolated from lower urinary tract infections (30%) or strains of fecal origin (12%). [10]. Correlating the hemolysin production with the presence of adhesins, we established that the hemolytic property was currently associated with MRHA rather than MSHA (Fig.2)

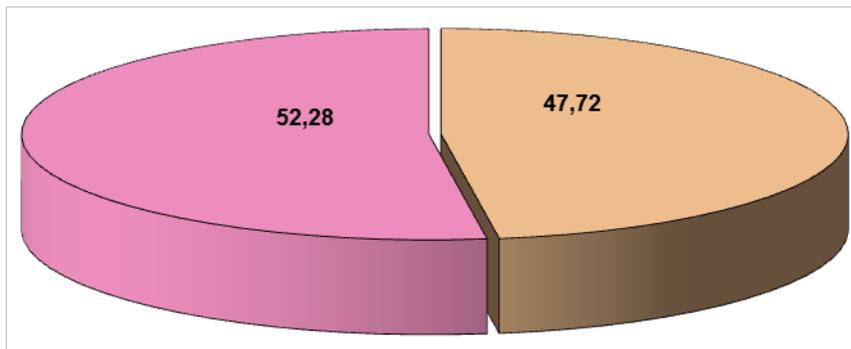


Fig.2. The correlation between mannose-resistant hemagglutination and hemolysin production

The incidence of hemolysin was found in 68 strains (32,70%) while the remaining 140 strains lacked this characteristic (67,30%). The positivity percentage is similar to the data provided by the literature, ranging from 38 % to 51 %. Out of the 68 strains which presented hemolysis, the incidence of those MRHA+ was significantly higher than the incidence of MSHA+, MRHA+ MSHA++ and hemagglutination free strains. The total amount of MRHA + strains correlated to the hemolysin production reached a point of 61,67 % (out of 68 hemolytic strains, 42 were MRHA+), the remainder 38, 24% of the hemolytic strains being MRHA- (out of the 68 hemolytic strains, 28 were MRHA)

Out of a total of 140 non-hemolytic strains, 46 were MRHA+ which is equivalent to 32,85 %, a much lower percentage than the one

registered for hemolytic strains. The data are consistent with those found in the works of J. Blanco and collaborators who reported a 79% rate of hemolytic and MRHA+ strains. [9] Genetic testing has shown that the genes for P fimbriae (the main fimbriae determining MRHA in urinary strains of E. coli) and the genes responsible for the production of hemolysin are closely related, their simultaneous appearance contributing to the strains uropathogenicity.

The production of cytotoxins in urinary E. coli and correlation with other pathogenicity factors

The 68 hemolytic strains of urinary E. coli were tested for the production of the cytotoxin, thus obtaining characteristic cytotoxic effect for 26 strains (38.20%) whereas its absence was registered in 42 strains (61.80%). - (Fig.3)

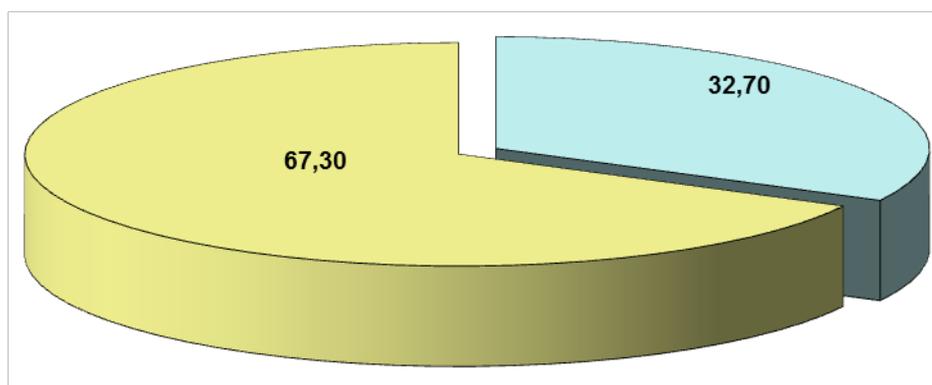


Fig.3. The cytotoxicity of hemolytic E.coli strains

In many studies, the incidence of CNF is closely correlated with the incidence of hemolysin in most populations of E. coli. The correlation of CNF with MRHA+, in urinary

hemolytic strains of E.coli, revealed that MRHA+ is consistently more frequent in Hly+ CNF- strains (66.66%) than in Hly+CNF+ strains (46,15%). (Table I)

Table 1. The association of hemolysin production , cytotoxin and the presence of adhesins

MRHA ⁺	HLy ⁺			
	CNF ⁺		CNF ⁻	
	No.	%	No.	%
	26	38,20	42	61,80
	12	46,15	28	66,66

The affiliation of urinary E.coli strains to the serogroups involved in UTI

It is well known the fact that the E.coli strains isolated from urinary tract infections belong to certain characteristic O serogroups ,the

most common being O2, O4, O6, O14, O22, O75, O83.

Regarding the 208 strains of E.coli isolated from urinary infections we established the belonging the serogroups O1-O25 and at the same time the affiliation to enteropathogenic serotypes. (Fig.4)

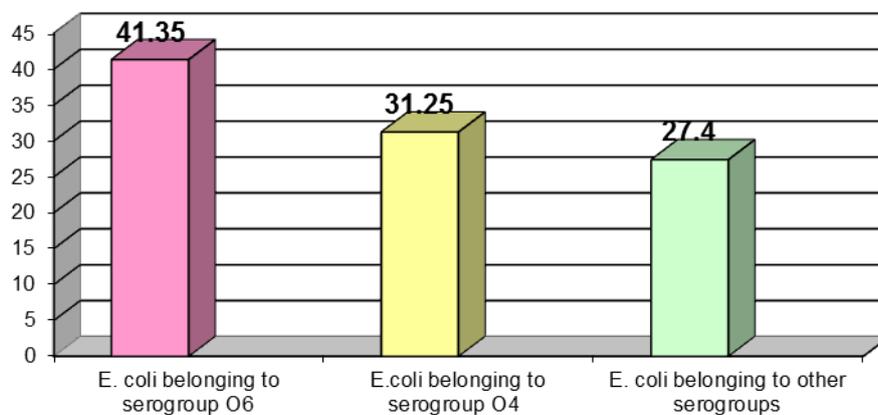


Fig.4. The appurtenance of E.coli strains to serogroups O1 – O25(%)

While following the membership of the studied strains to the serogroup O6 we established that it is more commonly related to the two groups of patients hospitalized versus

the outpatient group, a fact which advocates for the movement of this serogroup in hospital wards. (Fig.5)

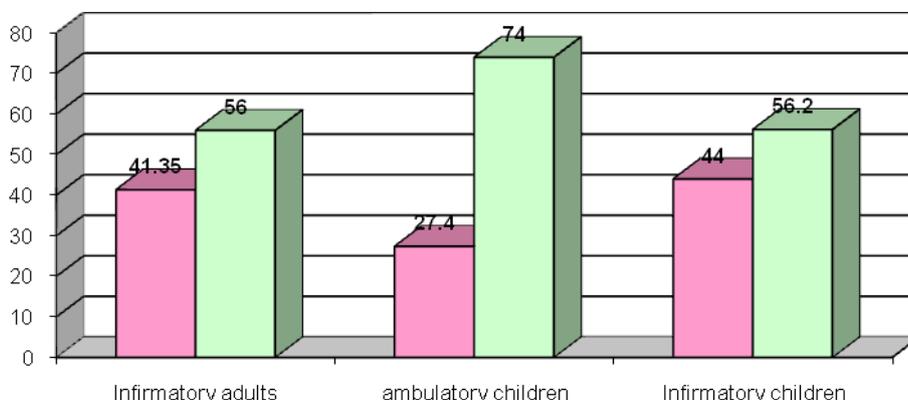


Fig.5. The susceptibility of serological group O6 to analysed patients

Specialty studies certify the result above, showing the frequency of serogroup O6 in E. coli strains isolated from nosocomial urinary tract infection (12.20%) and the presence of hemolysin in greater proportion in this serogroup.

Considering the data we obtained, 32 strains (37.20%) from the serogroup O6 were hemolytic up against the remainder serogroups (Table 2), hemolytic in proportion of 29,10%. Ulleryd and collaborators have reported that 73% of the strains isolated from pyelonephritis suffering men and 76% of the strains isolated from women with urinary tract infections presented

hemolytic activity. [5] Moreover, Yamada reported that 64% of E.coli strains isolated from men with prostatitis manifested a hemolytic phenotype.[11] Another finding of our study is the fact that E. coli O6 strains isolated from hospitalized adults are more frequently hemolytic than those isolated from the other groups (Table II) and MRHA was more common in hemolytic strains of the same group O6 (Table 3). The last observation is in agreement with the conclusion of Blanco and collaborators [9] regarding the fact that the genes encoding MRHA and hemolysis production are on the same chromosome in E.coli O6 and O4 strains.

Table 2. The hemolysin of E.coli strains belonging to O6 serogroup

The origin of strains	The amount of strains belonging to serogroup O	Hemolytic strains	
		No.	%
Infirmary children	18	4	22,22
Ambulatory Children	8	2	25
Infirmary adults	60	26	43,3

Table 3. The hemolysin and hemagglutination in E.coli belonging to O6 serogroup

	HLy ⁺ (32 O6 strains)		HLy ⁻ (54 O6 strains)	
	No.	%	No.	%
MRHA⁺	12	37,5	12	22,2
MSHA⁺	8	25	16	29,7
MRHA⁺ + MSHA⁺	6	18,75	8	14,8
HA Absence	6	18,75	18	33,3

By correlating the presence of hemolysin with cytotoxin produced by strains belonging to serogroup O6, we found that this serogroup is associated to Hly +CNF+ phenotype. Otherwise, out of the 32 strains affiliated to serogroup O6, 25 strains (78.12%) have the capacity to produce cytotoxin, an ability absent in the remaining 7 strains (21.88%). Blanco and collaborators determined that serogroups O2, O4, O6, O75 types were associated with E. coli with a Hly + CNF + phenotype (the most common being O4 and O6) and serogroups O1, O8, O18, D25, O86 associated CNF- Hly + phenotype. The correlation of the serogroup with some pathogenic properties allow better characterization of strains in light of the potential to cause pyelonephritis and consequences on health.

Conclusions

The etiology of UTI comprises a large etiologic spectrum, the production being assigned to germs from the Enterobacteriaceae family, E.coli being involved in the largest extent.

Not every germ and not at all times can enter into relationship with urinary structures. The presence of adhesion, hemolysin, and CNF are factors that confer uropathogenicity to germs.

Mannose-resistant hemagglutination is more frequent in strains that develop Hly but do not produce CNF, than in strains producing CNF. We ascertain the prevalence of serogroup O6 found in isolated strains from UTI, often in the hospital, being correlated with the Hly + CNF + phenotype.

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