MICROBE LANDSCAPE AND BIOLOGICAL PROPERTIES OF MICROORGANISMS REVEALED FROM THE URINE OF THE PATIENTS WITH THE UNCOMPLICATED INFECTIONS OF THE URINE TRACT

The study considers microbe landscape and basic microbiological characteristics of the revealed infectious agents in acute and chronic uncomplicated infections of the urological tract (UTUI). The E.coli species seem to be prevailed (66.3%) as etiological agent of the uncomplicated infections of urinary tract. The microorganisms of this kind were defined in monoculture in 78% of cases and in associations with the other microorganisms in 22%. The distinctions in frequency of isolation of E.coli strains, urine specie (Ur E.coli) and fecal specie (Kol E.coli), having only mannose-resistant hemagglutinins, and also combination of manno-resistant and mannose-sensitive hemagglutinins. Presence of only mannose-sensitive hemagglutinins with identical frequency were registered in the cultures E.coli, isolated from the urine of the patients with acute and chronic pyelonephritis and from feces of the healthy people.

Keywords: Microflora, urological tract, biological properties, manno resistant and mannose sensitive hemagglutinin, the patients.

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The infections of the urine tract are most widespread bacterial infections in all age groups (Dyadik and Kolesnik, 2003; Bacheller and Bernstein, 1997; Elmanama et al., 2006; Hombach et al., 2006). The rate of infections of the urine tract accounts for: 1% among newborns; 2-3% among children of preschool age (ratio boys/girls 1:10); 1-2% among the schoolboys (ratio 1:30); 2-5% among adult of the reproductive age (ratio men/women 1:50); 20-30% among elderly, in this age group the women are ill more often. Exception includes the hospitalized patients; their ratio is 1:1 (Dyadik and Kolesnik, 2003; Kondratova, 2005; Stapleton, 2006).

The etiology of the infections of urine tract has been studied in the plenty of researches. The infectious agents of the urological infections are the microorganisms, colonized the periurethral area. In many jobs it is noted that basically they are conditional pathogenic microorganisms of the family of enterobacteria, and E.coli is the leading among them (Dyadik and Kolesnik, 2003; Strachunskiy, 1999; Chew et al., 2006).

Now the urological infections are divided into uncomplicated and complicated. It defines the different approaches to the treatment. If the therapy of the uncomplicated infections of the urological tract has no special difficulties, the treatment of the complicated forms is a complex task. At first, because of pathogenic features (anatomo-functional disturbances); at second, because of etiology, in which an the resistant strains of bacteria play the important role including so-called “problem” - pseudomonas aeruginosa, atsinetobakter and others (Kondratova, 2005; Roos et al., 2006).

The occurrence of the infections of the urological system is the multifactor derivative. Study of the microbe landscape of the urine, microbiological characteristic of the activators of the inflammatory process, biochemical properties of microorganisms allows understanding special features of the disease development, causes of the resistance of microflora to antibacterial preparations and to choose correctly the therapeutic strategy.

However, till now many questions concerning this problem have been still unresolved. They include peculiarities of the qualitative and quantitative identification of the
conditional pathogenic and pathogenic microorganisms in patients with the infections of the urological tract.

In this connection, purpose of the present research was the study on microbe landscape and basic microbiological characteristics of the revealed infectious agents in acute and chronic uncomplicated infections of the urological tract (UTUI).

Material and methods

Totally, there were studied 600 urine samples obtained from the patients with UTUI. There were studied biological properties of 324 bacteria strains isolated from the urine of the patients with UTUI and 96 bacterial strains isolated from the bacteriological cultures of 380 urine samples of the outpatient patients who referred due to other diseases: acute respiratory diseases, cardiovascular pathology, the disease of the gastrointestinal tract (definition of the asymptomatic bacteriuria).

In total, we examined 420 patients at the age of 18-70 years with the uncomplicated infections of the urological tract from them 43 with acute and 72 chronic pyelonephritis, 109 with sharp and 100 with chronic cystitis, 96 with asymptomatic bacteriuria.

For performance of the put tasks the microbiological methods were used. All researches were carried out in the bacteriological laboratory of the of the Urgench branch of the Khorezm railway roads and at the chair of the microbiology and epidemiology of the Urgench branch of the Tashkent Medical Academy.

The biological properties were studied in all bacterial strains. Identification of microorganisms were made according to Bergey (1984). Deep bacteriological researches were performed with use of the standard methods concerning microorganisms isolated from the urine of the patients with bacteriuria, that is at CFU/ml (colony forming units in 1 ml of urine) more than 100 thousand of bacterial bodies ($10^5$>CFU/ml).

Biochemical activity was defined by cultivation in the semi-liquid medium containing various carbohydrates, ethanol and amino acids: glucose, lactose, mannitol, dulcit, arabinose, xyllose, citrate, acetate, malonate, phenylalanine, and lysine.

Antigenic structure of the studied cultures was established in reaction of agglutination on the glass with specific polyvalent and mono receptor results of the St.-Petersburg Scientific Research Institute of vaccine and serums (Russia).

Results and discussion

It is known, that quantitative and qualitative state of the normal microflora of the various biotopes of the body is not constant and varies under influence of various endogenic and exogenous factors. The quantitative increase of the representatives of normal microflora results in tension in the body immune system, as continuous antigen stimulating results in increase of functional loading of the immune system. It in turn results in occurrence of microorganisms in various biological liquids of the body, including urine. Bacteriuria shows the presence of pathological process in the body. Knowledge of the basic infectious agents of infections and their properties is important for performance of adequate chemotherapy of these infections and for their preventive maintenance.

Taking into account the above-stated, we present a spectrum of the selected microorganisms in all 420 studied patients (Table 1). For comparison of the results obtained we have given the landscape of microorganisms in relation to the gender, taking into account sexual differences of the identification of the infectious agents of the neuroinfections.

At clinically significant bacteriuria ($10^5$ CFU/ml and more) the infectious agent of infection was considered a bacteria isolated from the highest cultivation of urine. Clinically marked bacteriuria was established in 180 samples (30%) and clinically insignificant (less than $10^5$ CFU/ml) in 240 tests (40%). Others 180 (30%) urine tests appeared to be sterile.
TABLE 1. MICROORGANISMS ISOLATED FROM THE URINE OF THE PATIENTS WITH UNCOMPLICATED INFECTIONS OF THE UROLOGICAL TRACT, IN %

<table>
<thead>
<tr>
<th>Urine Microflora</th>
<th>Total number</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>59.2±4.5</td>
<td>63.6±2.4</td>
<td>56.7±3.8*</td>
</tr>
<tr>
<td>Hemolytic E.coli</td>
<td>7.1±3.5</td>
<td>8.1±3.4</td>
<td>6.5±4.3</td>
</tr>
<tr>
<td>K.pneumoniae</td>
<td>4.7±2.9</td>
<td>2.7±2.1</td>
<td>6.7±2</td>
</tr>
<tr>
<td>K.oxytoca</td>
<td>3.3±2.4</td>
<td>4.5±3.9</td>
<td>2±1.7</td>
</tr>
<tr>
<td>Enterobacter sp.</td>
<td>2.8±2.3</td>
<td>3.6±2.5</td>
<td>2±1.7</td>
</tr>
<tr>
<td>Pr.morganii</td>
<td>1.9±1.2</td>
<td>2.7±2.1</td>
<td>1±0.8</td>
</tr>
<tr>
<td>P.aeruginosae</td>
<td>1.4±0.8</td>
<td>0</td>
<td>3±2.4*</td>
</tr>
<tr>
<td>Gramnegative flora (totally)</td>
<td>80±3.1</td>
<td>86.3±3.9</td>
<td>77.7±4.2*</td>
</tr>
<tr>
<td>S.faecalis</td>
<td>3.8±3.3</td>
<td>5.5±3.3</td>
<td>3.2±1.2</td>
</tr>
<tr>
<td>S.epidermidis</td>
<td>5.9±4.3</td>
<td>8.2±2.2</td>
<td>5.1±2.3</td>
</tr>
<tr>
<td>Hemolytic S.epidermidis</td>
<td>2.4±1.9</td>
<td>4.5±1.9</td>
<td>1.6±0.8*</td>
</tr>
<tr>
<td>S. aureus</td>
<td>2.4±1.9</td>
<td>3.6±1.5</td>
<td>1.9±0.7</td>
</tr>
<tr>
<td>Hemolytic S. aureus</td>
<td>0.5±0.2</td>
<td>0</td>
<td>0.6±0.2*</td>
</tr>
<tr>
<td>S.haemolyticus</td>
<td>1.7±0.8</td>
<td>3.6±1.5</td>
<td>0.9±0.2*</td>
</tr>
<tr>
<td>S.saprophyticus</td>
<td>2.4±1.9</td>
<td>0</td>
<td>3.2±1.2*</td>
</tr>
<tr>
<td>S.viridans</td>
<td>1±0.8</td>
<td>1.8±1.7</td>
<td>0.6±0.2</td>
</tr>
<tr>
<td>Gramnegative flora (totally)</td>
<td>20±3.1</td>
<td>22.7±1.9</td>
<td>17.4±1.6*</td>
</tr>
<tr>
<td>Candida sp.</td>
<td>0.5±0.2</td>
<td>0</td>
<td>4.8±1.8*</td>
</tr>
</tbody>
</table>

Note: * - reliable differences of parameters of the women in comparison with the data of the men.

Is was established, that the etiological role of some kinds of gram negative bacteria in the women of reproductive age and men is different. So, from 310 strains selected from the women of reproductive age the great majority - 56.7±3.8% (176 women) were identified as E.coli. From strains of the other gram negative bacteria 6.7±2% were related to K.pneumoniae, 1±0.8% to Pr.morganii, 6.5±4.3% to hemolytic E.coli, 2±1.7% to Enterobacter sp., 3±2.4% to P.aeruginosae.

Among the strains of gram negative bacteria isolated from the men, E.coli strains accounted for 63.6±2.4%, Pr.morganii 2.7±2.1%, P.aeruginosae 0%, K.pneumoniae 2.7±2.1%, hemolytic E.coli 9.1±3.4%, Enterobacter sp. 3.6±2.5% (Figure 1).

![Figure 1. Comparative intersexual parameters of bacteria isolation from the urine of the patients with UTU](image-url)

The certain regularity was noted while studying gram positive microflora from the urine of the studied patients: in - first, gram positive microflora was isolated 4 times less, than gram negative microflora; in second, hemolytic S.epidermidis was revealed more in men, and hemolytic S.aureus in women; thirdly, coagulase-negative staphylococcus - S.epidermidis
and S.haemolyticus, and also coagulase-positive staphylococces - S.aureus were found more often men; in - fourth, at normal absence of isolation from the urine in men S.saprophyticus and Candida sp they were isolated in women in 3.2-4.8% of cases; in - fifth, the percent of isolation of gram positive flora in men was reliably more, than in women (22.7±1.9% vs. 17.4±1.6%).

The analysis of repeated microbiological investigations showed that in patients with aggravations of chronic pyelonephritis in 60% of cases the same kind of the infectious agent was preserved, and in 35% cases the change of a microorganism was noted. The frequency of the change of one kind of the infectious agent by another depended on the sex of patients: the change of microorganism was found in men in 50% of cases, and in women - in 30% of cases.

Most often E.coli was replaced by K.pneumoniae (30%) and by Proteus sp. (25%), less often by Enterobacter sp. (5%). In 5% of cases E.coli was replaced by not fermenting gram-negative bacteria and in 3% of cases by P.aeruginosae (Figure 2). Cases of the change K.pneumoniae by P.aeruginosae (10%) were also noted. The change of strain of the same kind of the infectious agent was revealed, predominantly in relation to drug resistance - in 50% of cases and on physiological-biochemical properties in E.coli in 40% of cases.

**Figure 2. Comparative intersexual parameters of bacteria isolation from the urine of the patients with UTUI**

![Diagram of bacterial distribution](image)

According to the current understanding in the basis of pathogenesis of the urogenital infections the process of adhesion of the microorganisms to the uroepithelial cells underlie. The adhesion of microorganisms is carried out by the fimbriae of the common type giving mannose-sensitive hemagglutination in the different mannose-resistant adhesins. The comparative analysis of the adhesive properties (colonization factors) of the uropathogenic E.coli (Ur E.coli) and isolators of fecal origin (Kol E.coli) showed that in relation to strains characterizing general hemagglutinating activity the bacterial cultures isolated from the urine of the patients with chronic pyelonephritis prevailed that in strains isolated from the urine of the patients with acute pyelonephritis and isolated from feces in practically healthy people (92.5%, 70%, 46.6%, respectively, p<0.001). In turn, quantity of the strains characterizing common hemagglutinating activity of E.coli, isolated from the
urine of the patients with acute pyelonephritis prevailed that in the strains isolated from feces in healthy subjects (70% and 46.6%, respectively, p<0.01).

There have been found differences in quantity of the E.coli strains, isolated from the urine and of fecal origin having only mannose-resistant hemagglutination as well as combination of mannose-resistant and mannose-sensitive hemagglutination with the same frequency registered in E.coli cultures, isolated from the urine of the patients with acute and chronic pyelonephritis and from feces of healthy people (Kol the E.coli-control).

The facts established have significant importance in diagnostics, prognosis of the development and outcomes as well as estimation of the uroinfection due to E.coli.

The profound bacteriological researches were performed in microorganisms isolated from the urine of the patients with bacteriuria, that is at CFU/ml (colony-forming units in 1 ml of urine) more than 100 thousand of microbe bodies (>105 CFU/ml). Etiological agent of UTUI in the studied patients E.coli (66.3%) were prevailed. The microorganisms of this kind were defined in monoculture in 78% of cases; in 22% of cases they isolated in associations with other microorganisms: in 12.0% cases with S.epidermidis and in 10% of cases with Candida sp.

Additionally to E.coli in the etiology of uroinfections the leading places were occupied by the representatives of family Entrobacteriaceae, Streptococcus sp., Enterococcus sp., Candida sp., S.epidermidis, P.aeruginosae.

The data obtained at the further identification of cultures, testify to uniformity of microorganisms of the sorts Escherichia, Pseudomonas, Staphylococcus on morphological, tinctorial and cultural properties.

The results of study of biochemical and some other properties of the strains Ur E.coli and Kol E.coli show, that cultures of studied strains utilize glucose, lactose, maltose, arabinose, mannit, sodium citrate and malonat, not fermentyze sorbit, ionosit, sodium cyrate and malonat, not produce serohydrarhium and acetylmetil carbinol. The have no beta-galactosiadase, urease, argindehydrolase, fenitalanindesaminase activity, but have enzymes lisin, and in the majority of cases ornitinudecarboxilase activity. According to the above-stated properties the strains analyzed did not differ from each other. At the same time, there were revealed strain differences in relation to ability to utilize sacarose. According to the results of the analysis a half of strains isolated from the urine of the patients with UTUI did not utilize sacaro.

Besides a part of strains isolated from the urine of the patients with chronic pyelonephritis and cystitis have no enzyme ornitin dekarboxylase. All studied strains have no hemolytic activity, except for the patients with chronic cystitis and chronic pyelonephritis.

At the same time the isolated cultures of microorganisms differed by variability on some biological signs in the frequency of the detection in them of the factors, to which the pathogenicity of bacteria was connected. E.coli strains showed variability by signs: maltose, sucrose and glucose fermentation, indol, protease, hemolytic and fibrinolytic activity formation as well as they were differed by antigen properties. The greatest number, from studied E.coli strains, belonged to the serogroup 01 (37% strains) and to serogroup 05 (20% strains).

The strains P.aeruginosae were variable in relation to formation of pyocyanin, urease, protease, fibrinolytic and hemolytic of activity.

The S.epidermidis species with various prevalence showed such signs as flocculation factor, fermentation of maltose, lactose and mannite, lecithinase and haemolytiic activity.

Thus, the UTUI infectious agents isolated from the patients with bacteriologically confirmed bacteriuria (105 CFU/ ml and are more), differ by variability of biochemical properties and by various frequency of sign manifestations, to which their pathogenesis is connected.
Conclusion

The E.coli species seem to be prevailed (66.3%) as etiological agent of the uncomplicated infections of urinary tract. The microorganisms of this kind were defined in monoculture in 78% of cases and in associations with the other microorganisms in 22% cases (in 12% of cases with S.epidermidis, in 10% of cases with fungi species Candida).

The distinctions in frequency of isolation of E.coli strains, urine specie (Ur E.coli) and fecal specie (Kol E.coli), having only mannose-resistant hemagglutinins, and also combination of mannose-resistant and mannose-sensitive hemagglutinins. Presence of only mannose-sensitive hemagglutinins with identical frequency were registered in the cultures E.coli, isolated from the urine of the patients with acute and chronic pyelonephritis and from feces of the healthy people.

The infectious agents of the uncomplicated infections of urine tract isolated from the patients with bacteriologically confirmed bacteriuria are differed by variability by some biological characteristics in the frequency of detection of the factors with which their pathogenicity is connected.

There have been revealed differences in adhesion of the strains E.coli, isolated from the various biospecies - urine and feces of the patients, feces of healthy people (Ur E.coli, Kol E.coli and Kol E.coli-control). The strains E.coli of urine origin have more expressed adhesive potential in comparison with E.coli of intestinal origin of the patients with the uncomplicated infections of urine tract and E.coli of the intestinal origin of the healthy people.

References