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Possibilities of bacteriophage therapy in the treatment of patients with complicated urinary tract infection

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Study aim*: evaluation of efficacy of a range of bacteriophages in children with urinary tract infection caused by a urologic pathology.* ***Study participants and methods****. The study involved 331 children with a urologic pathology manifesting itself with disturbed urodynamics and secondary urinary tract infection. 159 children received operative treatment; the other 172 children were subjected to conservative treatment methods. The patients were divided into 4 groups: (I) operative treatment + antibiotic therapy, (II) operative treatment + antibiotic therapy + bacteriophage therapy, (III) only antibiotic therapy, (IV) antibiotic therapy + bacteriophage therapy. Bacteriological urine culture was conducted. The revealed microbes were differentiated using 27 biochemical tests with subsequent determination of microbial sensitivity to antibacterial drugs. The trials were conducted at admission to inpatient hospital, 7-14 days after and 6-12 months after.* ***Results****. Enterobacteriaceae family pathogens (Escherichia coli) were prevalent at admission – 63.7%; Klebsiella spp. was the second – 15.2%; Pseudamonas aeruginosae – 9.2%, Enterococcus spp. and Enterobacter spp. – 6.0% each. Bacteriuria rate in the group I children was 38.5% at admission, 62.6% 7-14 days after and 32.5% 6-12 months after. There were no differences in preoperative and early postoperative periods in the group II children, but there was a tendency to bacteriuria rate reduction. The combined use of an antibiotic and a bacteriophage in children subjected to operative treatment resulted in an almost double bacteriuria rate reduction in comparison with a group of children who received only antibacterial therapy without an operation (5.8 and 13.7%, respectively). Differences in urine microfloral species composition in the children of groups III and IV indicate the reduction in hospital flora pathogens, especially in case of the combined use of an antibiotic and a bacteriophage: normalization of urine analyses within the first month in 81.3% of cases, remission duration longer than 6 months in 92.0% of cases.* ***Conclusions****: the use of bacteriophages appears to be prospective as antibiotic resistance of causative agents of various infections and of their complications increases.*

Keywords*: bacteriophage, bacteriophage therapy, urinary tract infection, urologic pathology.*

INTRODUCTION

The role of urinary tract infection (UTI) in the development of structural-functional renal disorders has been proven [1, 2]. UTI caused by disturbed urodynamics in the setting of congenital malformations of urinary tract organs, commonly known as “obstructive uropathy”, is especially severe [3]. Most patients require reconstructive-plastic operations, which determine the further course of an infectious process. Unless there is a pronounced renal dysfunction, conservative therapy, which is also aimed at normalizing urine passage, may be applied. At the same time, operative intervention itself is fraught with contamination by hospital flora, which may considerably aggravate the disease course. Antibacterial therapy is the only means of prevention and treatment in this situation [4]. However, this long-standing method has a range of disadvantages. First of all, it is allergization of the body, immunosuppressive action and development of dysbacteriosis, secondly – growth of microbial resistance to all known and widely used antibiotics, which provokes certain alarm in terms of resisting the infection in the future [5].

That is why practicing physicians constantly have to choose between antimicrobial action and intensity of side effects of antibiotics, especially when conducting therapy in postoperative period. Studies aimed at finding alternative and synergic methods of antimicrobial intervention have been conducted in recent years. One of such methods is the use of bacteriophages [6-9]. They had been discovered in the XIX century before antibiotics appeared.

Bacteriophages are viruses parasitizing on microbial cells. A big number of such viruses have been found for every bacterium. Some of them are highly pathogenic to microbial cells and cause lysis (lytic life cycle), the others are low pathogenic to bacteria (the so called phages with lysogenic, i.e. moderate life cycle) [10].

Bacteriophages do not cause negative side effects, inherent in antibiotics and many groups of antiseptics. They are strictly species- and type-specific and do not disturb natural microbiocenosis characteristics. At the same time, this group of drugs is successfully combined with traditional antibacterial drugs, thus increasing etiotropic effect; this allows using phage therapy in combined treatment of many inflammatory processes [11].

Unfortunately, bacteriophage therapy has not become widespread, apparently due to the historically established “reliability” of antibiotic therapy, conservatism of doctors and attitude of parents of ill children to doctoral prescriptions. It should be remembered that there have been no serious prospective randomized studies of the use of bacteriophages.

Study aim: evaluation of efficacy of the use of bacteriophages in children with UTI caused by a urologic pathology.

PATIENTS AND METHODS

Study participants

The study involved 331 children. All children had a urologic pathology manifesting itself with disturbed urodynamics in the form of ureteropelvic segment's obstruction (hydronephrosis), vesicoureteral segment's obstruction (megaloureter) and retrograde urine reflux from the urinary bladder to a kidney (vesicoureteral reflux). The study involved children with secondary UTI who had previously undergone antibacterial therapy domiciliary.

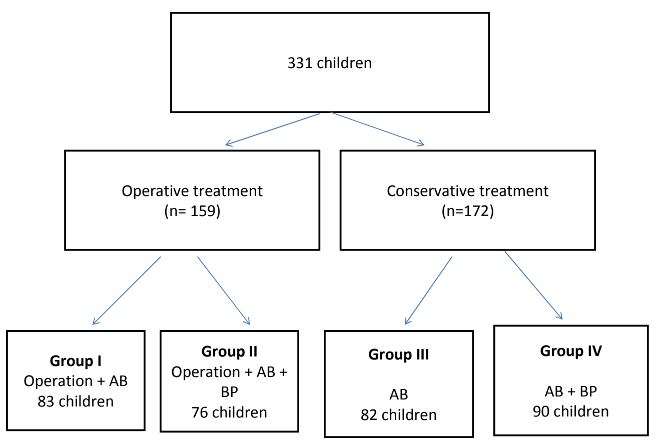
Study methods

Conservative and operative treatment methods aimed at normalizing urodynamics and arresting microbial-inflammatory process in the urinary system were applied depending on the functional condition of kidneys. 159 children received operative treatment; the other 172 children were subjected to conservative treatment methods – antibacterial (AB) drugs and bacteriophage therapy (BP).

As the primary study aim was to evaluate the efficacy of using bacteriophages in the treatment of complicated UTI, the children were divided into 4 groups on the basis of the provided treatment:

- **Group I** (n=83, **operation** + **AB**) **–** children who underwent surgical correction and who were applied only antibacterial therapy given the sensitivity of microbes determined by the bacteriological urine culture.
- **Group II** (n=76, **operation** + **AB** + **BP**) **–** children with surgical correction who received a combination of an antibiotic and a bacteriophage, also given the microbe revealed in urine.
- **Group III** (n=82, **AB**) – children without operative treatment who received only antibacterial therapy.
- **Group IV** (n=90, **AB** + **BP**) **–** children without operative treatment who received both antibiotics and bacteriophages (pic. 1).

Pic. 1. Division of children into groups-



Bacteriological urine culture was conducted by the generally accepted method. We adhered to the generally accepted methods and classifications (Bergey, 1984) in specific and generic identification of opportunistic pathogenic microbes. The revealed microbes were differentiated using 27 biochemical tests recommended by the International subcommittee on Enterobacteriaceae using microplates “Bacterial Identification cartiges” and an immune-enzyme analyzer “Quantrum” (USA) with subsequent determination of microbial sensitivity to antibacterial drugs.

Antibacterial therapy was prescribed given clinical recommendations at treating UTI and the data of local microflora monitoring regularly conducted in our center. The inhibitor-protected aminopenicillins served as the first-line drugs. Cephalosporins were used rarer; we used aminoglycosides and, extremely rarely, reserve group antibiotics – carbapenems – in case of severe UTI course in the setting of febrile temperature. We administered antibiotics step by step. The antibiotic was administered intravenously or intramuscularly in the first days until the febrile temperature decrease, then – per os in age-adequate doses.

Bacteriophages (Coliproteicum, Klebsiellae, Pseudamonas) were prescribed in the dose of 10-20ml TID for 5-7 days.

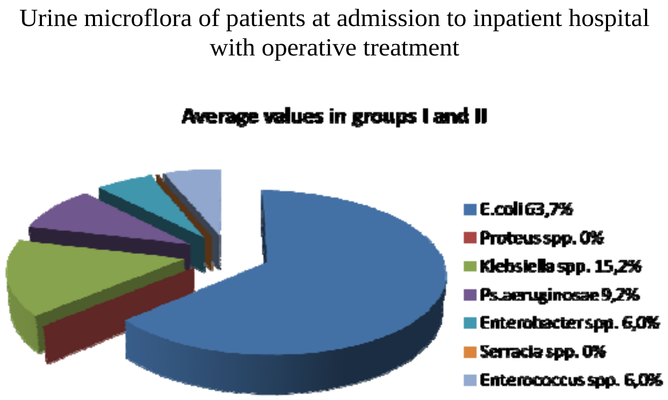
Bacteriophages were used locally after the urine culture results were obtained on the 7th day using operative drains set intraoperatively during surgical correction (pyelostomata, ureteral catheters, cystostomata). Pyelocaliceal cavity, urinary bladder cavity or ureteral lumen were locally irrigated by administering 5-10ml of the corresponding bacteriophage and clamping the drain for 15-20 minutes within the subsequent 5-7 days, removing the drain after that.

Evaluative criteria of effectiveness of the suggested treatment types were bacteriuria rate, duration of normalization of urine analyses, number of UTI exacerbations and remission duration. Microbial species composition was also evaluated. The trials were conducted at admission to inpatient hospital, 7-14 days (early postoperative period for the operated children) after and 6-12 months after (late postoperative period for the operated children and remote catamnesis for the children, who had been receiving conservative treatment).

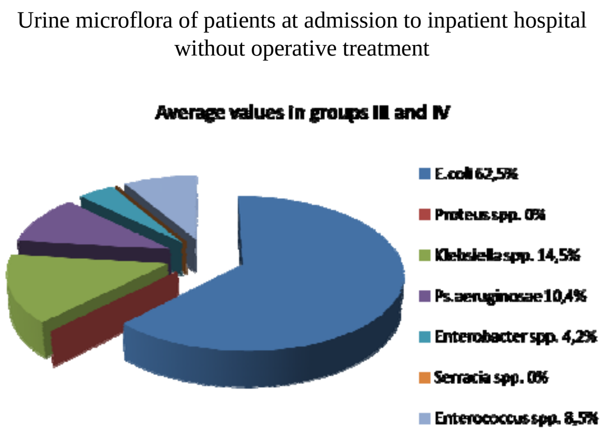
STUDY RESULTS AND DISCUSSION

Urine microflora of the patients, who received operative treatment during hospitalization, at admission is given in pic. 2. It appears that Enterobacteriaceae family pathogens (*Escherichia coli*) were prevalent – 63.7%; *Klebsiella spp*. was the second – 15.2%; *Pseudamonas aeruginosae* – 9.2%, *Enterococcus spp*. and *Enterobacter spp*. – 6.0% each. High share of *Klebsiella spp*. and *Pseudamonas aeruginosae* is caused by multiple invasive diagnostic or medical manipulations applied to children domiciliary before they were admitted to our center, which is why a hospital infection is present. It should also be remembered that many children were prescribed massive chronic antibiotic therapy; that is why the negative result of a bacteriological urine analysis did not always reflect the real course of the infectious process. This also explains the urine microflora of those patients who were treated using conservative methods. Their indices are almost the same as in the group of patients with operative treatment (pic. 3).

Pic. 2. Urine microflora of the patients (at admission to inpatient hospital) who were later subjected to operative treatment

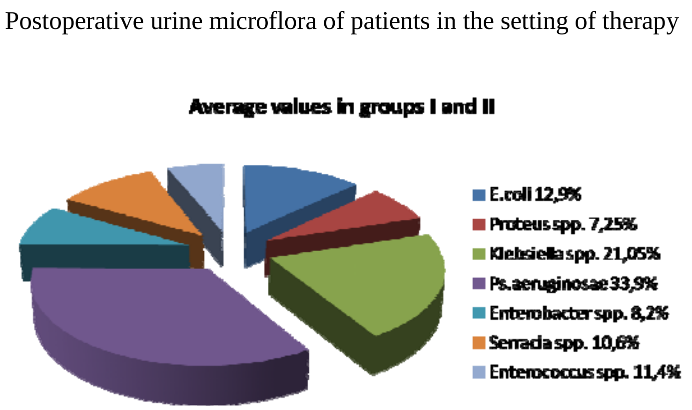


Pic. 3. Urine microflora of the patients (at admission to inpatient hospital) who were not later subjected to operative treatment

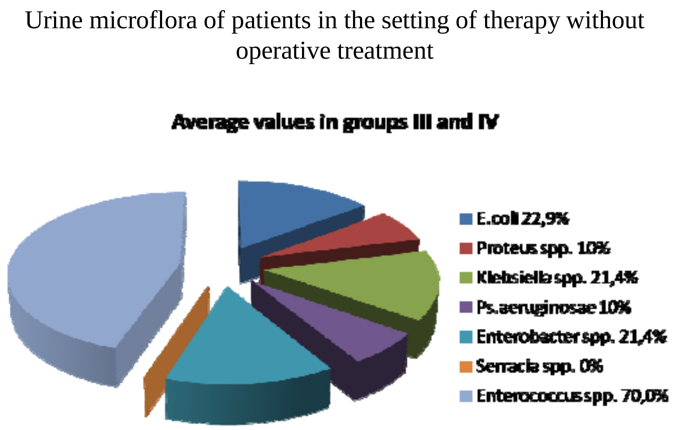


Microbial spectrum of causative agents changed in the process of treatment, mainly due to the increase in the share of hospital flora representatives in patients. This was most clearly revealed in patients with operative treatment methods. These indices are less in patients in those patients who were treated conservatively (pic. 4 and 5).

Pic. 4. Urine microflora of the patients after the operation in the setting of therapy



Pic. 5. Urine microflora of the patients without operative treatment in the setting of conservative therapy



Study of bacteriuria rate in the group I children showed that it was 38.5% at admission and increased up to 62.6% in the early postoperative period (7-14 days after the operation). The follow-up study of children 6-12 months later revealed bacteriuria in 32.5% of cases. Undoubtedly, bacteriuria rate increase in the early postoperative period is caused by the invasive character of the procedure and presence of hospital flora in the surgery and resuscitation units. However, we did not see a considerable bacteriuria rate decrease 6-12 months later, after urodynamics had normalized, in comparison with the value at admission to inpatient hospital (38.5 and 32.5%, respectively).

Comparative analysis of bacteriuria rate in the group II children did not reveal statistically significant differences in the preoperative and early postoperative periods; however, it recorded a tendency to bacteriuria rate decrease in this group's children in the early postoperative period. We see it as a positive aspect in the struggle against early postoperative complications, which are always more serious and take a severer course. Considerable bacteriuria rate decrease should be noted in the late postoperative period in the group II children in comparison with the group I children (7.9 and 32.5%, respectively). A similar regularity was revealed by the examination of children of the groups III and IV. Bacteriuria was revealed almost 2 times rarer (5.8 and 13.7%, respectively) when a combination of an antibiotic and a bacteriophage was used in the children who underwent operative treatment in comparison with a group of children who received only antibiotic therapy without an operation.

The use of an “antibiotic + bacteriophage” complex in the operated children led to the reproportioning of the urine-extracted microbes in the late postoperative period. Urine isolates in children who underwent an operation and received antibacterial therapy were represented by *Escherichia coli*, *Pseudamonas aeruginosae* and *Klebsiella spp*. (77.7, 7.5 and 14.8%, respectively), while only *E. coli* was registered in the late postoperative period in children who received an antibiotic and a bacteriophage – in 44.4% of cases.

Differences in urine microflora species composition in children of the groups III and IV indicate the reduction in the unit weight of hospital flora representatives, especially in case of the combined use of an antibiotic and a bacteriophage. The share of *Escherichia coli* was 20%; however, *Enterococcus spp*. was frequently revealed. Unfortunately, we could not use a bacteriophage against this microbe, as we did not have it (tb. 1).

The patients were divided into 2 groups regardless of the operative treatment for the comparative evaluation of the suggested therapy methods: the first group was comprised of the children who received only antibacterial therapy, the second – a combination of an antibiotic and a bacteriophage. A highly effective combination of an antibiotic and a bacteriophage was proven by the urine normalization duration, rate of UTI relapses and exacerbations. It remained highly effective even in case of the disturbed urodynamics and would become even more effective after it had been normalized by conservative or operative methods in comparison with the traditional antibacterial therapy. Urine analyses normalized within the first month in 81.3% of cases, remission duration longer than 6 months was 92.0% and there were no UTI exacerbations in 91.1% of patients within a year (tb. 2).

Table 1. Bacteriuria rate and urine microflora in children with complicated urinal tract infection

Groups of children and treatment types	Study period	Bacteriuria rate	Microbial species						
			E. coli	*Proteus spp.*	*Klebsiella spp.*	*Ps. aeruginosae*	*Enterobacter spp.*	*Serrasia spp.*	*Enterococcus spp.*
Operation + AB, n=83	preoperative	<u>32</u> 38.5%	<u>20</u> 62.5%	—	<u>5</u> 15.6%	<u>3</u> 9.5%	<u>2</u> 6.2%	—	<u>2</u> 6.2%
	7-14 days after	<u>52</u> 62.6%	<u>7</u> 13.3%	<u>3</u> 6.2%	<u>11</u> 21.1%	<u>18</u> 34.6%	<u>4</u> 8.0%	<u>6</u> 10.6%	<u>3</u> 6.2%
	>6-12 months	<u>27</u> 32.5%	<u>21</u> 77.7%	—	<u>4</u> 14.8%	<u>2</u> 7.5%	—	—	—
Operation + AB + BP, n=76	preoperative	<u>22</u> 28.5%	<u>14</u> 64.9%	—	<u>3</u> 14.7%	<u>2</u> 8.8%	<u>1</u> 5.8%	—	<u>1</u> 5.8%
	7-14 days after	<u>24</u> 31.8%	<u>3</u> 12.5%	<u>2</u> 8.3%	<u>5</u> 21.0%	<u>8</u> 33.3%	<u>2</u> 8.3%	—	<u>4</u> 16.6%
	>6-12 months	<u>6</u> 7.9%	<u>2</u> 44.4%	—	—	—	—	—	—
AB, n=82	preoperative	<u>23</u> 28.8%	<u>14</u> 61.0%	—	<u>3</u> 13.0%	<u>2</u> 8.7%	<u>1</u> 4.3%	—	<u>3</u> 13.0%
	7-14 days after	<u>14</u> 17.4%	<u>5</u> 35.8%	—	<u>3</u> 21.4%	—	<u>3</u> 21.4%	—	<u>3</u> 21.4%
	>6-12 months	<u>11</u> 13.7%	<u>4</u> 36.3%	—	<u>2</u> 18.1%	—	<u>3</u> 27.2%	—	<u>2</u> 18.1%
AB + BP, n=90	preoperative	<u>25</u> 27.9%	<u>16</u> 64.0%	—	<u>4</u> 16.0%	<u>3</u> 12.0%	<u>1</u> 4.0%	—	<u>1</u> 4.0%
	7-14 days after	<u>10</u> 11.3%	<u>1</u> 10.0%	<u>1</u> 10.0%	—	<u>1</u> 10.0%	—	—	<u>7</u> 70.0%
	>6-12 months	<u>5</u> 5.8%	<u>1</u> 20.0	—	—	—	—	—	<u>4</u> 80.0%

			%						

Note. Numerator – number of patients, denominator – percentage; AB – antibiotic therapy; BP – bacteriophage treatment.

Table 2. Dynamics of urinal tract infection (UTI) course in children by therapy types

Groups	Normalization period of urine analyses			UTI remission duration			Rate of UTI relapses			No exacerbations within 1 year
	1 month	3 months	6 months	1 month	3 months	6 months	Once a year	2-3 times a year	3 and more times a year	
Antibacterial therapy, n=165	28 17.0%	33 20.0%	104 63.0%	76 46.0%	40 24.0%	49 30.0%	30 18.0%	28 17.0%	18 11.0%	89 54.0%
Bacteriophage + antibacterial therapy, n=166	135 81.3%	19 11.6%	12 7.1%	—	13 8.0%	153 92.0%	15 8.9%	0	0	151 91.1%

Note. Numerator – number of patients, denominator – percentage.

Early postoperative period in children is characterized by operative drains. Although urine flows through them out to sterile one-time urinals, the risk of their infection by the microbes circulating in the environment is rather high. There is a term “catheter-associated” microflora, which means the formation of biofilm on the walls of urine drains. A biofilm is an accumulation of microbes and fragments of their nucleic acids in mycopolysaccharidic medium that jointly form a structured population on a hard surface. The microbes inside biofilms are well-protected against the mechanical effect of urine flow, other macroorganism’s protective factors and action of antibiotics. Traditional laboratory tests easily reveal free-floating bacteria in urine; however, fragments of bacteria located inside the biofilm structures do not grow on the standard growth media.

Despite the fact that drains remain in the body for only a short period (at the average, they are removed from urinary tracts after 7-14 days), this time is enough to contaminate urinary organs with hospital flora with subsequent chronization of the infectious process. Microbial species composition was the same in urine microflora taken from the operative drains. We revealed the same shares of *Kl. pneumoniae*, *E. coli*, *Ps. aeruginosae* and *Enterococcus* in operative drains in children regardless of how they received antibiotics – in the form of monotherapy or in a complex with bacteriophages (see pic. 4).

Given the presence of operative drains and, thus, of the possibility of direct local action on the microbial agent, we used the irrigation of urinary organs’ cavitary systems (urinary bladder cavity, pyelocaliceal complex, internal ureteral lumen) with bacteriophage solutions. The use of Coliproteicum, Klebsiellae and Pseudamonas bacteriophages that they are highly effective in case of local administration as well; it was proven by the bacteriological urine culture control. Thus, a Klebsiellae bacteriophage exterminated Kl. pneumoniae in 100% of cases, Coliproteicum bacteriophage was effective in 75% of cases, Pseudamonas – also in 75% of cases. Bacteriuria persisted even after the operative drains had been removed in children who received only antibacterial therapy, without local administration of bacteriophages (tb. 3).

Table 3. Efficacy of local administration of bacteriophages

Bacteriophage species	Number of positive results of bacteriological urine cultures	Number of negative results of bacteriological urine cultures	Efficacy
	Before treatment	After treatment	%
Klebsiellae	46	4	93.2
Coliproteicum	52	13	75.8
Pseudamonas	34	11	67.5

Despite the fact that bacteriophage therapy efficacy was appraised using the traditional antibiotic therapy (the study involved children in the setting of operative treatment, when perioperative antibiotic prevention is strictly necessary), the inclusion of these drugs convincingly proved advantages of their use.

CONCLUSION

Advantages of bacteriophages – simplicity of use and good tolerance – allow using them in small children. The use of bacteriophages appears to be prospective as antibiotic resistance of causative agents of various infections and of their complications increases.

Unfortunately, phage drugs are not widespread in clinical practice. The primary reason restricting their use is the insufficient clinical application experience and lack of doctoral knowledge of fundamental principles of treating with these drugs. In order to solve this problem, further clinical works based on the evidence-based medicine principle are required.

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