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ORIGINAL ARTICLE

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ORIGINAL ARTICLE

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MODERN TRENDS OF CHANGES IN THE MICROBIOTA OF UROGENITAL SYSTEM IN PATIENTS WITH UROLITHIASIS

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ABSTRACT

Aim: Analysis of trends in the microbial communities of the genitourinary system in patients with urolithiasis.

Materials and Methods: 165 urine samples from patients with urolithiasis was examined. The quantitative isolation of microflora was carried out using the bacteriological method. Microorganisms were identified using API biochemical test systems (bioMerieux, France). The percentage of the various types of microorganisms was determined. The reliability of differences in the frequency of various types of microorganisms isolation in a monoculture and the composition of microbial associations was determined by two-field tables with Fisher's Exact criterion. The GraphPad Prism 7 software was used.

Results: 198 bacterial cultures of various types were isolated. In one case, a *Candida* culture was isolated. *E. faecalis* was the most frequently isolated culture (29.1% of the isolated strains number); *E. coli* (18.1% of the total number of isolated cultures; *K. pneumoniae* (11.1%). There were no significant differences in the rate *of E. faecalis* compared to *E. coli*. *K. pneumonia* was isolated significantly less frequently than *E. faecalis*. These types of microorganisms were also leaders in the formation of bacterial mixes. In addition, these species are involved in the urease activity of bacteria (directly or indirectly), which contributes to the formation of stones in the genitourinary system.

Conclusions: *E. faecalis* is the species most often isolated from patients with purulent-inflammatory processes in patients with urolithiasis, both in the case of mono-infection and as part of mixed bacterial cultures.

KEY WORDS: *E. faecalis*, uropathogen, opportunistic infection, mixed infection

INTRODUCTION

Opportunistic infectious pathology is an urgent problem for modern medical science [1,2]. The pathogenicity factors of conditionally-pathogenic microorganisms are among the unsolved, actively studied questions [3], the mechanisms of their interaction with the affected cells and tissues [4]. In particular, for urolithiasis, the question of the microorganisms' contribution to the process of stone formation is essential [5]. The microbial in urolithiasis is currently in the process of active study and remains relevant. Moreover, being generally similar, the data presented by various authors have some differences. Maybe it is due to the influence on the study results of the characteristics of the examined groups of patients, nutrition, climatic conditions of people living in a particular country, level of medical care, primarily with the widespread use of antibiotics in medical practice.

AIM

Analysis of trends in the microbial communities of the genitourinary system in patients with urolithiasis.

MATERIALS AND METHODS

The studies were carried out in the bacteriological laboratory of Municipal Enterprise Poltava Regional Clinical Hospital, named after MV Sklifosovsky, Poltava, Ukraine, from 2020-to 2021. According to the Ethics Commission of Poltava State Medical University, the examination procedure for these individuals was conducted following the standards of the ethics committee. The material from patients was taken before the antibacterial therapy. The study was carried out of 165 samples of the middle portion of urine from patients diagnosed with urolithiasis. The bacteriological method was used following the normative and methodological recommendations [6]. The quantitative isolation of cultures was carried out according to the Gold method. In addition, the results in which the content of microbial cells was not less than 10^5 in 1 ml of urine was taken into account. The isolated microorganisms were identified using API biochemical test systems from bioMerieux (France).

The percentage ratio of various microorganisms from the number of isolated cultures and the number of investigated

Table 1. The frequency of selection of microorganisms from the urine taken from patients with urolithiasis

№	Groups, species	Total (number)	Sum (mono+mixes)	From the number of isolated strains (out of 199)		From among the studied samples (out of 165)	
				Total (%)	By groups (%)	Total (%)	By groups (%)
	Gram-positive bacteria						
	Staphylococci						
1	*S. aureus*	2/5	7	3,5		4,2	
	*S. epidermidis**	1/0	1	0,5		0,6	
2	*Coagulase-negative staphylococcus*	15/3	18	9,0	13,0	10,9	15,7
	Streptococci						
3	*S. agalactiae**	5/0	5	2,5		3,0	
4	*Bacteria of the genus Streptococcus*	9/0	9	4,5	7,0	5,5	8,5
	Enterococci						
5	*E. faecalis*	43/15	58	29,1		35,2	
6	*E. faecium*	8/3	11	5,5	34,6	6,7	41,9
	Other gram-positive bacteria						
7	*Corynebacterium spp.*	0/2	2	1,0	1,0	1,2	
	Total gram-positive bacteria				55,6	67,3	
	Gram-negative bacteria						
	Enterobacteria						
8	*E. coli*	28/8	36	18,1		21,8	
9	*K. pneumoniae*	10/12	22	11,1		13,4	
10	*P. mirabilis*	3/2	5	2,5		3,0	
11	*S. marcescens*	1/2	3	1,5		1,8	
12	*K. oxytoca*	1/1	2	1,0		1,2	
13	*Enterobacter**	2/0	2	1,0		1,2	
14	*Citrobacter*	1/1	2	1,0	36,2	1,2	36,9
	Non-fermenting bacteria						
15	*Acinetobacter*	6/3	9	4,5		5,5	
16	*P. aeruginosa**	2/0	2	1,0		1,2	
17	*Another non-fermenting bac.*	2/1	3	1,5	7,0	1,8	8,5
	Other gram-negative bacteria						
18	*H.parainfluenza***	0/1	1	0,5	0,5	0,6	
	Total				43,7	46,0	
	Fungi						
19	*Candida***	0/1	1	0,5	0,5	0,6	
	Total	139/60	199	99,8 (100)	99,8 (100)	100,0	

Notes: 1. Data are presented as fractions (X/Y), where the numerator represents the number of strains isolated in the monoculture and the denominator - from the mixes;
2. microorganisms were isolated only in monoculture;*
*3.** microorganisms were isolated only as part of mixed samples.*

samples was determined. The statistical reliability of differences in the frequency of isolation of microorganisms in monoculture and mixed cultures' composition was also determined. The frequencies of their obtaning were compared in two-field tables with the determining of Fisher's Exact criterion. The data were processed in absolute numbers. The GraphPad Prism 7 software was used.

RESULTS

Table 1 presents data of the frequency of various microorganisms isolation from the test material taken from patients with urolithiasis. 199 cultures of microorganisms were isolated in a bacteriological study of 165 urine samples taken from patients with urolithiasis (Table 1). 198 bacterial cultures and 1 culture of the *Candida* genus fungi were isolated. The predominant number of microorganisms belonged to gram-positive bacteria (staphylococci, streptococci and enterococci) and gram-negative bacteria (enterobacteria and gram-negative non-fermenting bacteria). In addition, in some cases, *Haemophilus parainfluenza* (1 case), *Corynebacterium* (2 cases) were isolated.

E. faecalis was isolated in monoculture in 43 cases and as part of mixed cultures – in 15 cases. 11 cultures of *E. faecium* isolates were also obtained (8 isolates in monoculture

and 3 in mixed cultures). Enterococci rate was 34.6% of all selected isolates. Moreover, it was exceeded the isolates of staphylococci (13%) and streptococci (7.0%) significantly.

Among the isolated gram-negative bacteria, the preponderate amount was represented by representatives of the *Enterobacteriaceae* family (36.2% of the total number of all isolated microorganisms). Half of this number were *E. coli*, isolated mainly in monoculture (28 cases). 8 straines were isolated from the mixed cultures. In total, *E. coli* accounted for 18.1% of the total number of isolated microorganisms.

K. pneumoniae was the following species on the frequency of occurrence (11.1%). In total, 22 isolates of bacteria of this species were isolated, of which 10 were in monoculture.

Thus, the dominant species turned out to be: *E. faecalis* were identified in 58 cases (29.1%), including 15 times in the mixes; *E. coli* (18.1%), *K. pneumoniae* (11.1%), coagulase-negative staphylococcus (9%), *E. faecium* (5.5%), bacteria of the genus *Streptococcus* and *Acinetobacter* (4.5% each), *S. aureus* (3.5%). In total, this amounted to 85.3%. The rest of the microorganisms accounted for 2.5% or less of the total cultures.

E. faecalis turned out to be the leader in participation in the mixed cultures (60.7%): in four cases with *E. coli*; 3 combinations each with *S. aureus*, *E. faecium* and *K. pneumoniae*. The remaining 4 cases are represented by single combinations of this microorganism with other species (in one of the cases, with *Candida* fungi). The second position was occupied by *K. pneumoniae* (9 cases). In addition to three mixes with *E. faecalis*, in two cases, combinations with *E. coli*, and in isolated cases with *S. aureus*, coagulase-negative staphylococci, *P. mirabilis* and *S. marcescens*. *E. coli* has been identified seven times in associations. In addition to four combinations with *E. faecalis*, two cases with *K. pneumoniae* and one case with *K. oxytoca*. *S. aureus* was the fourth most frequently involved in mix formation (5 cases). In 3 cases out of 5 identified, these were mixed with *E. faecalis*. In fifth position (4 cases each) were *E. faecium* and coagulase-negative staphylococci. Moreover, for coagulase-negative staphylococci, these were combinations with various microorganisms (*E. faecium, K. pneumoniae, S. marcescens, P. aeruginosa). E. faecium,* on the contrary, was found in three cases in combination with *E. faecalis* and only in one – with coagulase-negative staphylococcus.

When the mixed mixture consisted of 3 different microorganisms in all three cases, the microbial association included *E. faecalis* and *K. pneumoniae*. The variable portion was represented by *E. coli, Acinetobacter* and *Alcoligenes faecalis*.

DISCUSSION

Uropathogens are represented primarily by species that usually inhabit the human intestine. However, the microbiome excreted from the urine of patients with urolithiasis correlates to a greater extent with the microbiome isolated directly from stones and much less – with the species composition of microorganisms isolated from the urine of patients without urolithiasis [4]. Disruption of the genitourinary system microbiome, usually not accompanied by disorders of the intestinal microbiome [7]. In addition, the genitourinary microbiome, to a greater extent than the gut microbiome, is subject to changes due to antibiotic use [7].

The list of "classic" uropathogens is represented in the literature primarily by gram-negative bacteria – *E. coli* (45-80%), as well as *Proteus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*. A lesser role is given to gram-positive bacteria – bacteria of the genus *Staphylococcus*, including coagulase-negative species (*S. saprophyticus* and *S. epidermidis*), *Streptococcus* group *D, Enterococcus*. Among the anaerobic forms of bacteria, it is customary to call bacteroids. Today *Streptococcus agalactiae, Aerococcus, Citrobacter kosery, Enterobacter, Serratia*, capnophilic bacteria, *Actinomyces, Prevotella, Corynebacterium urealyticum*, *Gardnerella, Actinobacilum* (subclass *Actinomycetes*), *Candida albicans* are listed as potential uropathogens. The existing trend towards increasing the frequency of *E. faecalis* from patients with urolithiasis is associated with the widespread use of antibiotics in modern medical practice [10].

The data of this study essentially correspond to the generally accepted ideas about the species composition of microorganisms isolated from patients with urolithiasis. At the same time, as a result of our statistical analysis, for the first time, a significant quantitative predominance of *E. faecalis* over *E. coli,* which was in the second position in terms of the number of isolated strains was found ($P < 0.05$). It was especially true of *K. pneumoniae*, which occupies the third position in terms of the number of isolated cultures and other, less abundant species and groups of microorganisms.

The altered microbiome may directly contribute to the process of stone formation [8]. Particular importance is attached to the presence of urease activity of microorganisms secreted from patients with urolithiasis. It is believed that this enzyme is directly related to the formation of stones. However, urease activity is not always a property necessary to ensure the vital activity of producing bacteria; therefore, this property should be attributed to pathogenic factors capable of protecting microorganisms from the immune factors of the human body [5]. *E. faecalis* is not one of the bacteria producing urease, but it is characterized by associated with other urease-forming bacteria. It has been shown that in associations, *E. faecalis* has a potent effect, enhancing the manifestation of the pathogenic properties of other members of the association and promoting the formation of biofilms. The following two species, prevailing among cultures isolates from pathological material – *E. coli* and *K. pneumoniae* – may produce urease [10].

Thus, the examples of mixed infections identified in our study are consistent with the literature data. The analysis of the frequencies of isolation of microorganisms in a monoculture and the composition of microbial associations in two-field tables with the determining of Fisher's exact criterion was carried out for the cultures most often isolated from patients: *E. faecalis, E. coli* and *K. pneumoniae*. The statistical analysis results showed no significant differences in the propensity to form microbial

associations between *E. faecalis* and *E. coli* (P> 0.05). On the other hand, *K. pneumoniae*, which was isolated from the composition of associations more often than in monoculture, is significantly more (P <0.05) inclined to be present in the composition of associations than *E. faecalis* and *E. coli*. Such microorganisms and their combinations were found, contributing to the formation of stones in the genitourinary system. The same three species of bacteria were included in 5 species isolated from the composition of three-component associations found in six study cases. Moreover, in three out of six samples, both *E. faecalis, E. coli,* and *K. pneumoniae* were present. At the same time, some species were isolated only in monoculture (*S. epidermidis, S. agalactiae, Enterobacter, P. aeruginosa*), and others only in mixed-species (*Haemophilus parainfluenza, Corynebacterium*).

CONCLUSIONS

E. faecalis is the most numerous species isolated from patients with purulent-inflammatory processes in patients with urolithiasis. *E. coli* and *K. pneumoniae* occupy the second and third positions in the list of cultures isolated from pathological material taken from patients with urolithiasis. The indicated species are also leading in their participation in the bacterial mixes. It applies to both two-component and three-component microbial associations.

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CONFLICT OF INTEREST

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