ORIGINAL ARTICLE

MICROBIOLOGICAL ANALYSIS OF ABDOMINAL CAVITY EXUDATE, BLOOD AND AFFECTED TISSUES SAMPLES FROM PATIENTS WITH INTRA-ABDOMINAL ABSCESSES IN COMPLICATED INFECTION OF ABDOMINAL CAVITY

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ABSTRACT

The aim: To conduct an analysis of the results of a microbiological examination of biological samples taken from patients with intra-abdominal abscesses.

Materials and methods: Material for microbiological examination was collected from 60 patients during surgery and transported to laboratory at the same day. Isolation and identification of microbial pure cultures were performed by standard microbiological methods. Statistical analysis was performed using Statistica software.

Results: Analyzing the microbiological research results indicated importance of the sample collecting time (first or repeated surgery). In patient's blood taken during first surgery it was found a statistically significant predominance of no growth of microflora. In abdominal cavity exudates anaerobic cultures increased statistically significantly in repeated surgery. It was noted that in samples taken during first surgery mixed pathogens were represented mainly by facultative anaerobic cocci, then in repeated surgery anaerobic microorganisms were predominant. Examination of liver abscess content found that monoculture was isolated in 85.7 %. Blood and affected tissue samples in such patients were sterile. Investigation of samples from patients with multiple abdominal cavity abscesses revealed anaerobic microorganisms in 16.7 %. Blood samples of that patients in 40 % were sterile.

Conclusions: An analysis showed that in appendicular abscesses content gram-negatives were predominant. Gram-positive bacteria dominated in paravesical abscesses with 65 % isolates from gallbladder and 66.7 % from the affected tissue samples. In liver abscesses gram-positive cocci were isolated in 57.1 %. In multiple abdominal abscesses due to bowel perforation rod-shaped microflora was predominant (76 %) and represented by either obligate aerobes or obligate and facultative anaerobes.

KEY WORDS: microorganisms, liver abscess, paravesical abscess, gram-negatives, anaerobes

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INTRODUCTION

Intra-abdominal abscesses (IA) are considered to be one among serious problems in abdominal surgery [1]. The frequency of IA in the overall structure of surgical diseases is high. Among the many causes of IA, the main is hematogenous deposition of the pathogen from primary inflammatory focus, frequently from an inflamed abdominal organ [2]. Currently, due to the significant increase in the incidence of inflammatory diseases of the gallbladder and biliary tract, a significant amount in the overall structure of IA are liver abscesses and paravesical abscesses [3]. It is not always possible to establish the exact time of appearing of liver and paravesical abscesses, as they are usually preceded by long-term, often recurrent purulent cholangitis [4]. Abscesses that develop due to the transition of inflammation from the gallbladder to the liver, also usually appear on the background of prolonged cholecystitis [5].

Increasing in the frequency of surgeries on abdominal cavity with leaving drainages and stents for a long time also contributes to the rise in the number of IA [6].

Among the reasons for hematogenous IA are septic conditions of various genesis, acute purulent inflammatory diseases of the abdominal cavity, less often - infectious foci of other localization, including furuncules, hydradenitis, infected wounds that lead to rapid formation of abscesses in the abdominal cavity. In some cases, the direct cause of IA may not be reliably established [7].

Reseach data are unsystematic and sometimes contradictory, so microbiological analysis of samples from IA will not only help in qualitive study of the microflora, also in de-

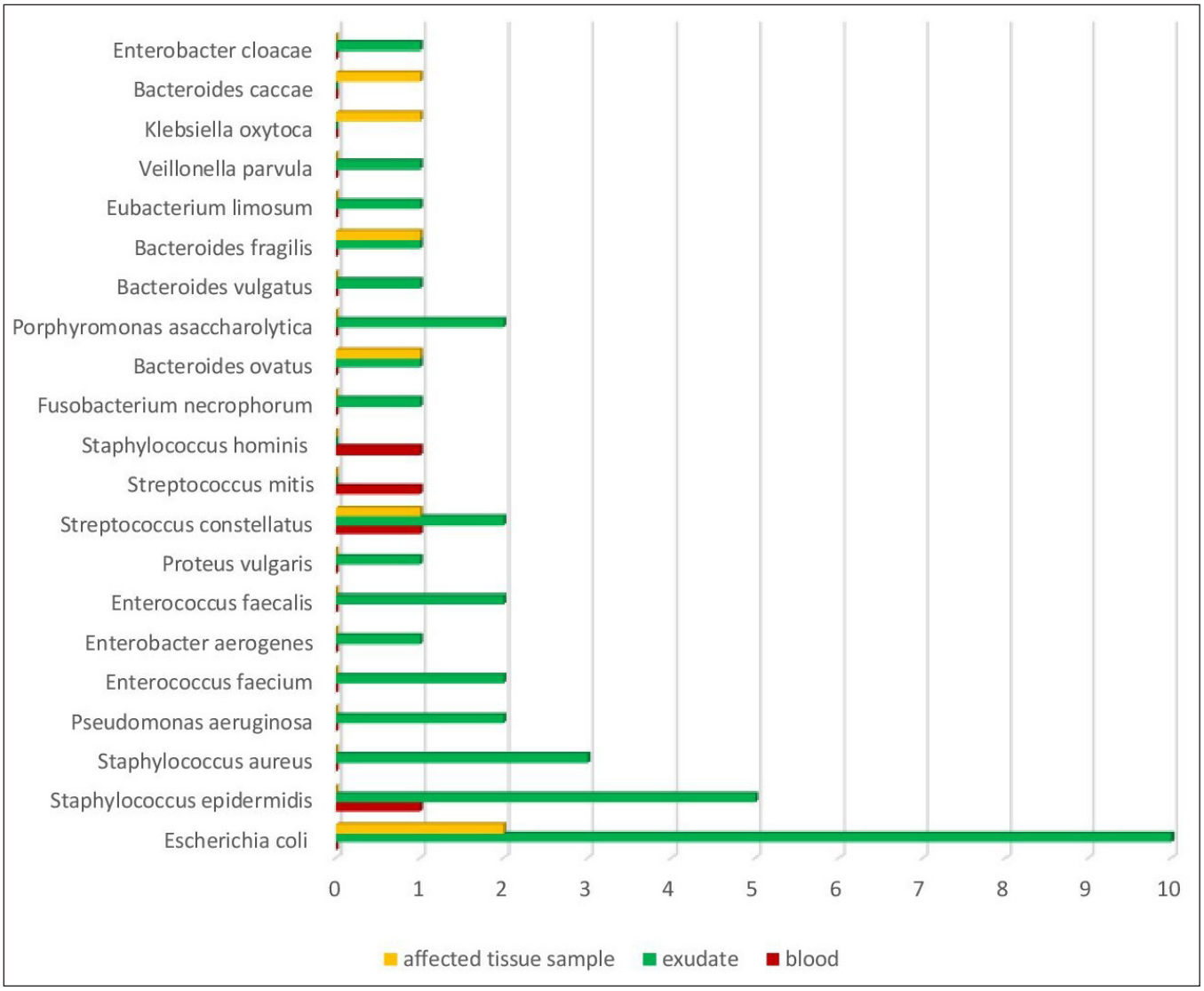

Fig. 1. The range of etiological factors (absolute number of strains) isolated from the samples of patients with appendicular abscesses.

tecting unusual pathogens such as *Streptococcus mitis* and *Streptococcus oralis*, which can cause severe complications.

THE AIM

The aim of research was to conduct an analysis of the results of a microbiological examination of biological samples taken from patients with intra-abdominal abscesses in complicated infection of abdominal cavity.

MATERIALS AND METHODS

Material for microbiological examination was collected from patients with intra-abdominal abscesses in complicated infection of abdominal cavity that were treated in the surgical department of Kharkiv City Hospital № 18 during period from 2015 to 2020. 60 patients with intra-abdominal abscesses were included in analysis and divided into 4 groups. 20 patients with appendicular abscesses formed group I; 25 patients with ruptured gallbladder, paravesical abscesses were in group II; 10 patients with liver abscess were in group III; 5 patients with multiple abdominal abscesses due to bowel perforation formed group IV. Material such as abscess content, abdominal exudate, affected tissues samples and blood was taken during surgery in patients and transported to laboratory at the same day. Isolation and identification of microbial pure cultures were performed by standard microbiological methods using Micro-la-tests (Czech Republic). Statistical analysis was performed using Statistica Software (StatSoft Inc., version 9, 2009 USA). All groups undergone the Gaussian distribution model. We established that the distribution in each group was abnormal. Methods of nonparametric statistics were used.

RESULTS

It was found that in samples from group I patients with appendicular abscesses (abdominal cavity exudate, af-

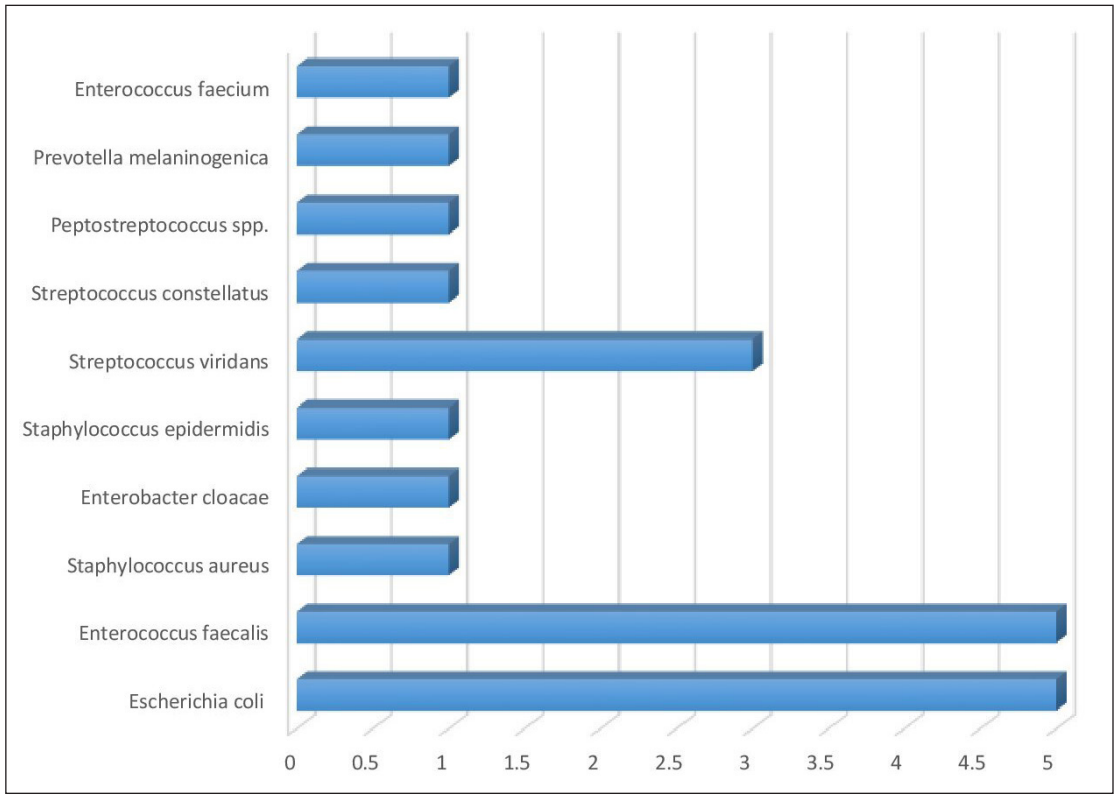

Fig. 2. The range of etiological factors (absolute number of strains) isolated from gallbladder content of patients with paravesical abscesses.

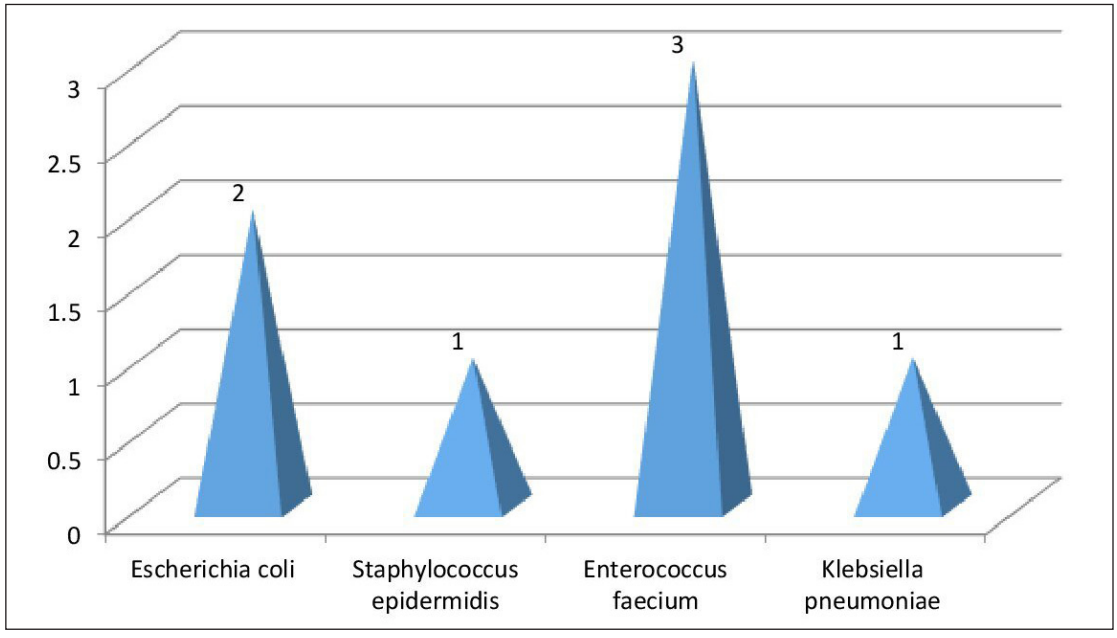

Fig. 3. The range of etiological factors (absolute number of strains) isolated from abscess content of patients with liver abscesses.

fected tissues and blood received within 24 hours after surgery) 48 strains of microorganisms were isolated (Fig. 1). From abdominal exudate predominantly (55 %) mixed cultures of *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Enterococcus faecium, Streptococcus constellatus* and *Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae/aerogenes, Proteus vulgaris* together with *Porphyromonas asaccharolytica, Fusobacterium necrophorum, Bacteroides ovatus, Bacteroides vulgatus, Bacteroides fragilis, Eubacterium limosum, Veillonella parvula* were detected. From affected tissues *Streptococcus constellatus, Escherichia coli, Klebsiella oxytoca* and *Bacteroides caccae, Bacteroides fragilis, Bacteroides ovatus* were isolated. It should be noted that only from one sample pathogens were not isolated. From blood of two patients (10 % of

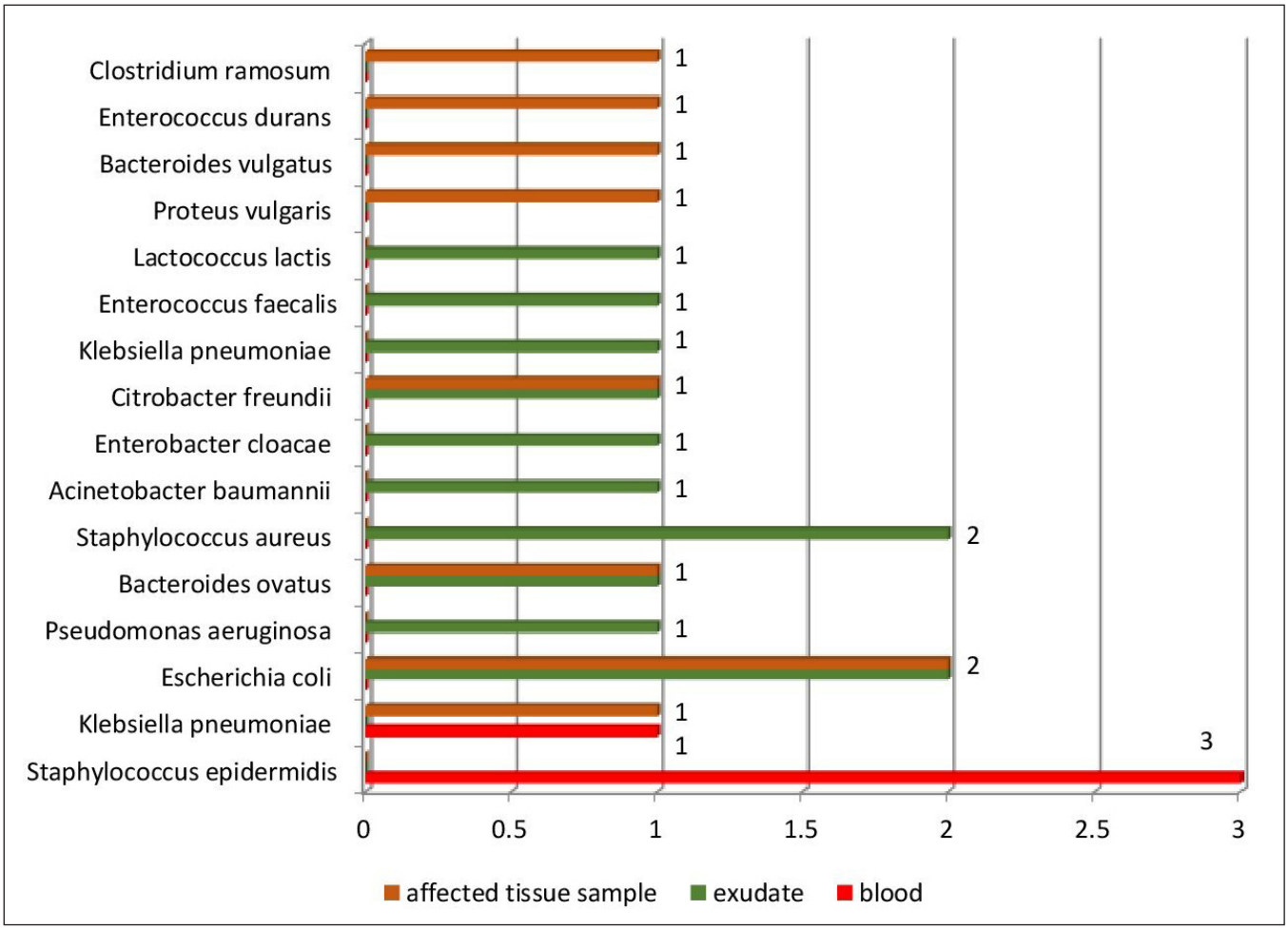

Fig. 4. The range of etiological factors (absolute number of strains) isolated from patients with multiple abdominal abscesses during investigation of blood, exudates and affected tissue samples.

cases) facultative anaerobic gram-positive cocci were identified such as *Streptococcus constellatus* in monoculture and mixture of *Streptococcus mitis*, *Staphylococcus epidermidis* and *Staphylococcus hominis*.

Control analysis on second day after surgery did not reveal microorganisms in blood of group I patients (sterile blood).

Only in one case in female that underwent surgery due to abscess on the 15th day after surgery mixed culture was identified in abdominal cavity exudate consisting of facultative anaerobic gram-positive cocci (*Streptococcus constellatus*) and anaerobic gram-negative rods (*Porphyromonas asaccharolytica, Fusobacterium necrophorum, Bacteroides ovatus*).

On the 4th day after repeated surgery in this patient from the abdominal cavity exudate facultative anaerobic gram-positive cocci (*Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis*) were isolated.

Therefore, 37 strains of microorganisms were isolated from abdominal exudates. In 5% of samples microorganisms were not detected. Cocci were isolated in 45.9%, rod-shaped microorganisms were detected in 54.1% of samples. In 21.6% anaerobes were cultured. From samples of affected abdominal tissue gram-positive cocci were revealed in 14.3%; anaerobic cocci and rods were detected in 42.9% of cases.

Investigation of group I patients blood samples found that growth of microorganisms was not observed in 18 (90 %) cases. In two patients, 4 microorganisms belonging to the facultative anaerobic cocci were detected in blood samples. Therefore, *Streptococcus mitis* is a typical pathogen of oral cavity, and its isolation from blood may be recognized as transitory bacteremia after tracheal intubation of the patient.

Investigation of abscess content from patients with gall bladder perforation, paravesical abscesses (group II) revealed 23 strains of microorganisms (Fig. 2). Gram-positive cocci were representatives of genera *Streptococcus*, *Staphylococcus*, *Enterococcus* and *Micrococcus*. This samples contained cocci in both mono (in 7 patients out of 12) and mixed culture, which was represented by both cocci and rods (in 3 patients). Rods predominantly were facultative anaerobes from family

Table I. Distribution of isolated microbial stains

Microorganisms (n=103 bacterial strains and 1 strain of yeasts)		1st group (n=48)	2nd group (n=23)	3rd group (n=7)	4th group (n=25)
isolated from	blood	4	-	-	4
	exudate	37	20	8	12
	tissue	7	3	-	9
gram-positive cocci	blood	4	-	-	3
	exudate	17	13	4	3
	tissue	1	1	-	1
gram-positive rods	blood	-	-	-	1
	exudate	1	-	-	1
	tissue	-	-	-	1
gram-negative rods	blood	-	-	-	1
	exudate	20	7	3	8
	tissue	6	2	-	7
obligate anaerobes	blood	-	-	-	-
	exudate	5	1	-	2
	tissue	3	2	-	2
Candida albicans	abscess content	-	-	1	-
mono-culture	blood	1	-	-	2
	exudate	7	12	6	1
	tissue	-	-	-	-
mixed culture	blood	1	-	-	1
	exudate	12	3	1	3
	tissue	2	2	-	2
Total (103 bacterial strains) exudate (n=37) tissue (n=7)		blood (n=4)	blood (n=0)	blood (n=0)	blood (n=4)
		gall bladder content (n=20)	abscess content (n=7)	exudate (n=12)	
		tissue (n=3)	tissue (n=0)	tissue (n=9)	

Enterobacteriaceae (*Escherichia coli, Klebsiella oxytoca*). The growth of representatives of *Pseudomonadaceae* and *Lactobacillaceae* families was also obtained. It was found that representatives of all families mostly were isolated in a mixed culture in the amount of $1x10^7$ CFU and were represented by facultative anaerobic gram-positive cocci (*Streptococcus constellatus, Staphylococcus aureus, Enterococcus faecalis, Enterococcus faecium, Streptococcus viridans*), anaerobic gram-positive cocci (*Peptostreptococcus spp.*), anaerobic gram-negative rods (*Prevotella melaninogenica/oralis*) and facultative anaerobic gram-negative rods (*Escherichia coli, Enterobacter cloacae/aerogenes*). 40 % of gallbladder samples were sterile, in other samples in 65 % cocci were detected. Obligatory anaerobic microorganisms were revealed in 5 % of cases.

The results of inoculation of affected tissues samples showed that in one case gram-negative anaerobic *Prevotella melaninogenica/oralis* in the amount of $1x10^3$ CFU in monoculture was detected. In two cases mixture of facultative-anaerobic gram-positive cocci (*Streptococcus constellatus*) and anaerobic gram-negative rods (*Prevotella melaninogenica*/oralis) in the quantity of $1x10^7$ CFU were isolated. It should be noted that in 92 % of cases pathogens were not revealed from the mentioned samples. All tested blood samples of group II patients were sterile.

Examination of liver abscess content in group III patients revealed 7 strains of bacteria (Fig. 3) and 1 strain of *Candida albicans* ($1x10^6$ CFU). Also it was found that in 30 % of cases the growth of microorganisms was not observed. Monoculture was isolated in 85.7 % of cases and represented by facultative anaerobic gram-negative rods *Escherichia coli* (2 strains in $1x10^8$ CFU) and *Klebsiella pneumoniae* (1 strain in $1x10^5$ CFU), gram-positive cocci *Staphylococcus epidermidis* (1 strain in $1x10^8$ CFU) and *Enterococcus faecium* (3 strains in $1x10^8$ CFU). Blood and affected tissue samples of group III patients were sterile.

Investigation of samples from patients with multiple

abdominal cavity abscesses (group IV) identified 25 strains of microorganisms (Fig. 4). From abdominal cavity exudates 12 strains of bacteria were isolated and in 20 % of cases the growth of microorganisms was not detected. Anaerobic microorganisms were found in 16.7 % and for the first time in the mixed culture obligate aerobic microorganism *Acinetobacter baumannii* (in $1x10^3$ CFU), a gram-negative proteobacteria of the family *Moraxellaceae* was found.

Gram-positive facultative anaerobic microflora was represented by *Staphylococcus aureus* ($1x10^{2-3}$ CFU), *Enterococcus faecalis* ($1x10^6$ CFU) and anaerobic bacteria *Lactococcus lactis (*$1x10^6$ CFU). Gram-negative facultative anaerobic bacteria included *Escherichia coli* ($1x10^3$ CFU and $1x10^6$ CFU), *Klebsiella pneumoniae* ($1x10^6$ CFU), *Citrobacter freundii (*$1x10^6$ CFU), *Enterobacter cloacae* ($1x10^8$ CFU). Also non-fermentive gram-negative aerobic rods *Pseudomonas aeruginosa* ($1x10^3$ CFU) and gram-negative anaerobic rods *Bacteroides ovatus (*$1x10^6$ CFU) were isolated.

From affected tissue samples of two group IV patients 9 strains of bacteria in mixed culture were revealed. It was found that the main etiological factors were gram-negative facultative anaerobic rods (2 strains of *Escherichia coli* in $1x10^3$ CFU and $1x10^8$ CFU; 1 strain of *Klebsiella pneumoniae* in $1x10^8$ CFU; 1 strain of *Citrobacter freundii* in $1x10^6$ CFU; 1 strain of *Proteus vulgaris* in $1x10^5$ CFU); gram-negative anaerobic rod-shaped bacteria *Bacteroides vulgatus* ($1x10^6$ CFU) and *Bacteroides ovatus* ($1x10^6$ CFU) and gram-positive obligate anaerobic rods *Clostridium ramosum* ($1x10^6$ CFU). Gram-positive facultative anaerobic cocci were represented by *Enterococcus durans* ($1x10^7$ CFU).

In blood samples of group IV patients 4 strains of bacteria were identified (table I), and it was found that in 40 % it was sterile. Isolated bacteria were 3 strains of *Staphylococcus epidermidis and 1 strain of Klebsiella pneumoniae.*

DISCUSSION

There are research data about determination of intra-abdominal abscesses etiological factors, which indicates that the main causative agents are purulent microbes - streptococci, staphylococci and rarely anaerobic microorganisms. IA can also been as the result of infecting with enterobacteria, enterococci and gram-negative flora [8-10]. According to data of recent years *Klebsiella pneumoniae*, *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumani* and *Proteus spp.* are frequently isolated from patients with IA.

The growth of microorganisms in the bacteriological study of IA content is detected according to various authors in most patients, but seriously depends on the timing and rational use of antibacterial therapy [11]. In addition, the spectrum of microorganisms that cause purulent lesions in the abdominal organs depends on the way of pathogen's penetration. Thus, in liver abscesses gram-negative microorganisms with a predominance *Escherichia coli* up to 24 % and gram-positive cocci with a predominance of *Enterococcus spp.* up to 17 % are on the first place. The frequency of detection of non-clostridial anaerobic flora in patients with liver abscesses can reach, according to experts, up to 41.8 %. There are reports indicating that approximately in a half of patients, especially those who undergo antitumor chemotherapy, staphylococci with multi-drug resistance are isolated [12].

The microflora in acute appendicitis complicated by abscess is represented by mixed cultures of facultative aerobes and obligate anaerobes [13]. And the most common are gram-negative bacteria, *Escherichia coli* and *Bacteroides fragilis* [14]. Our research showed that gram-positive flora, most often *Enterococcus faecalis* was predominant and found in 65 % of the abscess content and 66.7 % of the affected tissues. Gram-positive cocci, mainly *Enterococcus faecium*, were isolated in 57.1 % of liver abscesses. In multiple abdominal abscesses due to intestinal perforation, the rod-shaped flora was predominant (76 %) and represented by both aerobes (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*) and obligate aerobes (*Bacteroides ovatus*).

Ratio M. and others [15] showed in their research data about 14 isolated microorganisms. In our research gram-negatives (mainly *Escherichia coli*) and gram-positive cocci (*Enterococcus spp., Staphylococcus spp.*) were predominant.

The fact that needs further analysis is the isolation from the blood (in two cases) of facultative anaerobic gram-positive cocci (*Streptococcus constellatus* in monoculture and *Streptococcus mitis, Staphylococcus epidermidis* and *Staphylococcus hominis* in mixed culture). On our opinion, it can be explained by the fact that blood for microbiological examination was collected after tracheal intubation during surgery.

Analyzing the results of microbiological research indicated importance of the sample collecting time (first or repeated surgery). In patient's blood taken during first surgery it was found no growth of microflora in most of all seedings.

In abdominal cavity exudates the number of cultures of anaerobic microflora and mixed culture seriously increased in repeated surgery.

It was noted that in samples taken during first surgery mixed pathogens were represented mainly by facultative anaerobic cocci, then in repeated surgery

samples anaerobic microorganisms were predominant, and rod-shaped pathogens have always been a part of mixed cultures.

CONCLUSIONS

Bacteriological analysis of intra-abdominal abscesses content in patients with acute abdominal infection showed that gram-negatives (54.1 %), most often *Escherichia coli*, were predominant in appendicular abscesses. Gram-positive bacteria dominated in paravesical abscesses with 65 % isolates from gallbladder and 66.7 % from the affected tissue samples, most often it was *Enterococcus faecalis*. In liver abscesses also gram-positive microorganisms were predominant (57.1 %), but *Enterococcus faecium* was most often. In multiple abdominal abscesses due to bowel perforation rod-shaped microflora was predominant (76 %) and represented by either obligate aerobes or obligate and facultative anaerobes.

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The Authors declare no conflict of interest.

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