PYOGENIC LIVER ABSCESS - ETIOLOGICAL SPECTRUM AND ANTIMICROBIAL SUSCEPTIBILITY

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ABSTRACT

INTRODUCTION: Pyogenic liver abscess (PLA) is a serious challenge in modern medical practice. The aim of this study was to investigate the etiology and antimicrobial susceptibility of PLA-associated microbial pathogens, diagnosed in hospitalized patients at St. Marina University Hospital of Varna during the period between 2001 and 2016.

MATERIALS AND METHODS: A total of 84 clinical samples (pus aspirates, n=72, bile samples, n=7, and blood cultures, n=5), collected from PLA patients, hospitalized in the Second Surgery Clinic were analyzed. Species identification was performed by conventional methods. Antimicrobial susceptibility was studied by disk diffusion method and Phoenix 100 (BD). The results were interpreted according to CLSI and EUCAST standards.

RESULTS: Causative bacterial agents belonging to 15 different species were isolated in 59 cases (in 70%). *E. coli* (23.7%), *K. pneumoniae* (20%), *E. cloacae* (13.5%), *E. faecalis* (8.5%), *P. mirabilis* (5%) and *P. aeruginosa* (5%) dominated in the etiological spectrum. *E. coli* demonstrated the following levels of antimicrobial susceptibility: imipenem, amikacin, piperacillin/tazobactam, 100%; ceftazidime, cefepime, 90.9%; cefuroxime, 83.3%; amoxicillin/clavulanic acid, 77.8%; gentamicin, 75%; levofloxacin, cefalothin, 66.6%; ciprofloxacin, 63.6%; piperacillin, 58.3%; ampicillin, 45.5%. The following antimicrobial susceptibility rates were determined for *K. pneumoniae*: imipenem, 100%; amikacin, 92.3%; ceftazidime, cefepime, 80%; cefalothin, cefuroxime, 75%; levofloxacin, gentamicin, 66.6%; ciprofloxacin, 62.5%; piperacillin/tazobactam, 57.1%; piperacillin, 25% and amoxicillin/clavulanic acid, 22.2%.

CONCLUSIONS: *E. coli* and *K. pneumonia* are the predominant pathogens in PLA patients. Carbapenems are the most active antimicrobial agents followed by ceftazidime and cefepime. In the aminoglycoside group, amikacin demonstrates the best *in vitro* activity. Scr Sci Med. 2017;49(4):53-57

Keywords: pyogenic liver abscess, *E. coli*, *K. pneumoniae*, antibacterial agents, resistance

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Received: October 14, 2017

Accepted: December 11, 2017

INTRODUCTION

Pyogenic liver abscess (PLA) is associated with inflammatory focus formation in the liver with a subsequent formation of pus-filled cavity. The disease is a serious therapeutic and surgical problem. At the beginning of the last century, pyelephlebitis associated with appendicitis is the most common cause of PLA and in 75-80% of the cases, the outcome of this infection is lethal. In the late 20th century to date,

both benign and malignant biliary tract obstructions are the most common cause for liver abscesses. Mortality has now declined significantly and ranges from 10 to 40% (1). To a large extent, this is due to the early diagnosis of the disease, adequate microbiological diagnosis and improved treatment options, including etiological antibacterial therapy.

According to literature data, PLA incidence rate has not changed significantly during the past 50 years - an average of 8 to 16 cases per 100 000 patients, with an autopsy rate ranging from 0.3 to 1.5% (2).

In developed countries, most liver abscesses are bacterial. PLA incidence rate is significantly lower with amoebic, mycotic and necrotic (sterile) etiology. The causative infectious agents can be identified by microbiological analysis of liver punctate, bile and blood cultures. The cultivation of bile and pus samples from the collected liver punctate is usually positive and allows the identification of the etiological agent in a high percentage of the cases, whereas a bacterial growth in the tested blood cultures is detected in 55-60% only (3).

The appropriate use of antibacterial therapy is essential for the successful outcome of the disease. Information about the etiological spectrum and antimicrobial susceptibility of the key organisms responsible for PLA significantly helps the surgeon and is an integral part of the complex approach to resolving this complicated therapeutic problem.

The aim of this study is to present data on the etiological spectrum of bacterial causative agents of PLA in patients hospitalized in the Second Surgery Clinic at St. Marina University Hospital of Varna during the period between 2001 and 2016 as well as their susceptibility to antimicrobial agents.

MATERIALS AND METHODS

During this period, a total of 84 clinical samples collected from PLA patients, hospitalized in the Second Surgery Clinic were examined in the Microbiology Laboratory of St. Marina University Hospital of Varna. The distribution of the tested clinical samples was the following: pus aspirates from liver abscess, n=72, bile samples, n=7, and blood cultures, n=5. Species identification was performed by conventional tests, Crystal and Phoenix 100 Identification Systems (BD). Antimicrobial susceptibility was studied by Kirby-Bauer disk diffusion method and Phoenix

100 (BD). The results were interpreted according to CLSI and EUCAST standards.

RESULTS

Causative bacterial agents were isolated in 59 cases (in 70%). Twenty-five clinical samples remained sterile. A mixed bacterial infection (involving more than one bacterial species) was identified in four patients. A total of 59 non-duplicate bacterial isolates belonging to 15 different species were isolated. Data regarding PLA etiological spectrum were shown in Table 1.

Table 1. Bacterial species isolated from patients with PLA (in %)

Microbial species	Isolates	
	Number	%
Gram-positive bacteria		
<i>Staphylococcus aureus</i>	2	3.4
<i>Staphylococcus epidermidis</i>	4	6.8
<i>Staphylococcus haemolyticus</i>	1	1.7
<i>Streptococcus anginosus</i>	1	1.7
<i>Enterococcus faecalis</i>	5	8.5
Gram-negative bacteria		
<i>Escherichia coli</i>	14	23.7
<i>Klebsiella pneumoniae</i>	12	20.3
<i>Klebsiella oxytoca</i>	2	3.4
<i>Leclercia adecarboxylata</i>	1	1.7
<i>Enterobacter cloacae</i>	8	13.6
<i>Citrobacter freundii</i>	1	1.7
<i>Proteus mirabilis</i>	3	5.1
<i>Pseudomonas aeruginosa</i>	3	5.1
<i>Pseudomonas putida</i>	1	1.7
<i>Bacteroides fragilis</i>	1	1.7
total	59	100.0

The susceptibility of the most frequently isolated species (*E. coli* and *K. pneumoniae*) to a set of 14 antibacterial agents was shown in Fig. 1 and Fig. 2.

DISCUSSION

PLA is a relatively rare, but potentially life-threatening disease. Rapid diagnosis, identification of etiologic agents and the early start of adequate medical and/or surgical treatment are among the most important factors for a therapeutic success.

Fig. 1. Susceptibility of *E. coli* isolates (n=14) to a set of antimicrobial agents

Fig. 2. Susceptibility of *K. pneumoniae* isolates (n=12) to a set of antimicrobial agents

Taken into consideration the specificity of pathogenesis of this disease, the leading etiologic agents of PLA are bacteria from the normal gastrointestinal microbial flora. In this aspect, the family *Enterobacteriaceae*, and particularly *E. coli*, which is repeatedly documented in a number of studies, has a dominant role (4-7). A retrospective study involving 72 patients with *E. coli* PLA found out that most often, the etiology of infection is associated with biliary tract pathology (8). These authors reported a relatively high mortality rate, mainly related to underlying malignancies, the presence of multiple abscesses and/or prolonged hypoalbuminemia. The results of our study indicate that *E. coli* is the most common bacterial pathogen and confirm its leading role in PLA etiology.

In recent years, mainly in the Asian countries, but also in some centers in USA and Europe, a trend of increasing the relative rate of PLA associated with *K. pneumoniae* was detected (9-11). A retrospective study from China identified *K. pneumo-*

nae as the primary bacterial pathogen of PLA in the period 2010-2015 as detected in 60% of the cases (9). The major reasons for this trend are not fully elucidated yet, however, underlying diseases such as diabetes mellitus, different individual susceptibility to infections caused by this species, expression of specific virulence factors and more frequent colonization with *K. pneumoniae* in some regions are considered of major importance (12). Some authors report significantly lower mortality rate in patients with *K. pneumoniae* PLA (13). The development of distant extrahepatic complications such as endophthalmitis, meningitis and necrotizing fasciitis is a typical clinical manifestation of this infection (14-17). Our own data show a high relative share of *K. pneumoniae* in the etiological spectrum, occupying the second place as a causative agent of PLA, but extrahepatic complications have not been found out in any of the patients.

In addition to these results, we detected a significantly lower relative share of Gram-positive bacteria. In this group, enterococci are the most common isolates (Table 1). Although their frequency is relatively low compared to that of Gram-negative bacterial species, the clinical significance of enterococcal infections is high because of their natural resistance to multiple antibiotics. The primary resistance of enterococci to all cephalosporins and clindamycin, as well as the acquired resistance to some antimicrobial agents such as amino- and ureidopenicillins, fluoroquinolones and glycopeptides, makes the adequate choice of an antibacterial therapy extremely problematic.

The obligate anaerobic microbial flora plays an important role in the development of intra-abdominal infections. Only a single anaerobic isolate (*Bacteroides fragilis*) was detected in all the studied clinical samples and it was isolated from a blood culture. It is noteworthy that a microbiological analysis for obligate anaerobic bacteria was performed only for blood cultures, rather than for purulent secretions and bile samples. This fact explained the low rate of anaerobic bacteria in the etiological spectrum of PLA and did not reflect the real significance of these bacteria for PLA development.

Polymicrobial associations are a common finding in PLA patients. Some authors report a relatively

high proportion of such infections - of 16.2% (4,18). Among the tested clinical samples, we found evidence of mixed infections with the involvement of two or three different bacterial species in four purulent samples collected from PLA patients (7.5%). The presence of more than one bacterial causative agent further complicates the adequate choice of antimicrobial therapy.

Data from the antimicrobial susceptibility testing of the most common bacterial agents of PLA (*E. coli* and *K. pneumoniae*) indicate that only carbapenems (imipenem, meropenem) and piperacillin/tazobactam (with respect to *E. coli*) demonstrate fully preserved *in vitro* antibacterial activity. The broad spectrum of activity of carbapenems, their excellent anti-anaerobic effect and good tissue penetration define these antibiotics as an excellent choice for treatment, especially in high-risk patients and in the cases of multidrug resistant bacteria, which are often associated with nosocomial infections. To preserve the excellent activity of carbapenems, it is necessary to evaluate the potential risk factors for the patient and to determine the resistance profile of the particular bacterial pathogen before starting the antibiotic therapy.

In the group of cephalosporins, ceftazidime and cefepime demonstrate the highest activity against both species *E. coli* and *K. pneumoniae*. Resistance to third- and fourth-generation cephalosporins among enteric bacteria is often related to the production of extended-spectrum β -lactamases (ESBL). According to our results, ESBL-producing isolates associated with PLA are relatively rare (9.1-20%), which allows the empirical treatment with ceftazidime and cefepime to be a component of the therapeutic approach in PLA patients. In a study comparing the etiological spectrum and antimicrobial resistance of the causative pathogens associated with PLA in patients with and without diabetes mellitus, a statistically significantly higher proportion of ESBL-producing *E. coli* and *K. pneumoniae* was established (18).

In the group of aminoglycosides, amikacin demonstrates a very good *in vitro* activity against these two predominant bacterial species - *E. coli* and *K. pneumoniae*. Because of the low level of resistance to amikacin (7.7% for *K. pneumoniae* and 0% for *E. coli*), it can be recommended as a part of the treat-

ment regimen of PLA patients. However, the lack of activity of the aminoglycosides against obligate anaerobic bacteria eliminates the option to use these agents as a mono-therapy. They can be used only as a part of combined antimicrobial treatment.

CONCLUSIONS

Our study demonstrates that Gram-negative microbial flora represented mostly by *E. coli* and *K. pneumoniae* clearly dominates in the spectrum of PLA-associated bacterial pathogens. The treatment with third- or fourth-generation cephalosporins such as ceftazidime and cefepime or piperacillin/tazobactam, in combination with amikacin results in most cases to a therapeutic success due to the low levels of resistance to these antimicrobial agents. Carbapenem therapy in high-risk PLA patients and in cases of suspected nosocomial infections is recommended.

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