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Microbiology of Liver Abscesses and Its Correlation with Hospital Stay

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Abstract: Pyogenic liver abscess (LA) is a rare disease associated with high morbidity and mortality rates, and prolonged hospital stay. Certain microbiological agents have assumed a predominant role in Asian series, however, few studies have been published in Europe regarding the microbiological agents involved in liver abscesses and their relationship with prognosis and length of hospital stay. The aim of this study was to characterize the main microbiological agents involved in LA in a European hospital, to assess resistance patterns and to relate them to patient prognosis and length of hospital stay. A retrospective analysis was conducted on all LA-diagnosed adult patients, admitted to a northern Portuguese hospital between 2013 and 2018. Clinical, laboratory, imaging, and microbiological data were collected for descriptive and statistical analysis. A total of 63 LA diagnosed patients were admitted to the General Surgery Unit between January 2013 and December 2018. Patients´ mean age at diagnosis was 71.4 years. Abdominal pain and fever were the most common symptoms on admission (73.0 and 61.9%, respectively). Fifty-eight percent of patients were female. Leukocytosis and increased C-reactive protein were the most observed analytical changes. The most frequently isolated microbiological agents were *Escherichia coli* (36.5%), *Streptococcus species* (27.8%), *Klebsiella pneumoniae* (11.4%), and anaerobic agents (10.1%). Of the isolated species, 12.5% proved multi-resistant. A higher LA frequency caused by *Klebsiella pneumoniae* was identified in relation to other Western series. *Klebsiella pneumoniae* was associated with a longer hospital stay (25.67 vs 16.50 days, p=0.07) when compared to other agents. There were 4 mortality cases in our series (6.3%). Microbiological agents, namely *Klebsiella pneumoniae* and multidrug-resistant agents have a predominant role in LA management, negatively affecting prognosis and length of hospital stay. Despite advances in LA treatment, more studies are required to determine the appropriate therapy owing to the absence of internationally defined guidelines. Our results provide important information for the proper management of these patients.

Keywords: Liver Abscess, Pyogenic Liver Abscess (PLA), Microbiology

1. Introduction

A pyogenic liver abscess (LA) is defined as an encapsulated collection of purulent material in the liver parenchyma. In developed countries, LA is a rare disease (2.3-100,000) [1, 2] associated with high morbidity and mortality rates (10% - 40%) [1] as well as prolonged hospital stays. Early diagnosis is crucial for LA treatment. However, its diagnosis may be challenging owing to the subtle and non-specific nature of symptoms. Thus, a high suspicion index is required for timely diagnosis. Drainage, percutaneous or surgical, and antibiotic therapy are the mainstays of treatment [3].

According to the literature, most frequently involved *microorganisms include Escherichia coli, Enterobactariaceae, Anaerobes* and others usually present in the gastrointestinal flora [4-6]. *Klebsiella pneumoniae* is a

predominant pathogen in Asia, and its rate has increased in the West [1-3, 6-8]. However, scanty studies explore LA microbiological agents in Europe. To fill the gap, the authors carried out a retrospective study of patients diagnosed with LA and admitted to a northern Portuguese hospital between 2013 and 2018. The study aimed to characterize the main microbiological agents involved in LA, according to LA etiology and to assess resistance patterns and relate them to patient prognosis and length of hospital stay.

2. Methods

The search for patients diagnosed with LA was conducted in the hospital database (*S. Clínico Hospitalar*). The search involved entering the diagnostic codes of the International Classification of Diseases-9 (ICD-9 572 and 572.0) between 2013 and 2016, and the most recent homologous version, ICD-10, between 2017 and 2018 (k75.0). Inclusion criteria for the diagnosis of LA were based on clinical, imaging, and microbiological criteria, using the following assumptions:

1. Any patient with imaging characteristics, compatible with an intra-parenchymal liver abscess, associated with elevated inflammatory markers,
2. Any patient with a positive microbiological culture of an intra-parenchymal liver drainage product.

Exclusion criteria:

1. Patients under 18 years,
2. Extra-parenchymal LA,
3. Abscesses following cholecystectomy (in cholecystectomy *locus*),
4. Non-pyogenic abscesses (amoebic and fungal),
5. Hydatid cysts,
6. Patients whose treatment did not include abscess drainage,
7. Rehospitalizations due to LA.

2.1. Data Collection

Laboratory, microbiological, and imaging data of patients were collected using the SClínico Hospitalar platform and associated applications (ClinidataNet and Syngo Plaza). Collected data included clinical data (admission date, discharge date, days of hospital stay, age, gender, previous comorbidities, symptoms on admission, diagnosis on admission, etiology of liver abscess, type of drainage performed, antibiotics performed, morbidity, and mortality data), laboratory data (analytical study on admission, before, and after drainage), imaging data (size, quantity and location of abscesses), and microbiological data (blood cultures and cultures of LA drainage product).

2.2. Clinical Data

The research team followed the Delphi Method to define LA etiology. LA were considered to be of biliary etiology in patients with a recent diagnosis of cholangitis, recent instrumentation or known alteration of bile ducts. Hematogenous dissemination was considered if another source of infection liable to hematogenous dissemination had been identified previously to LA diagnosis. The portal etiology was admitted in cases of intra-abdominal infection susceptible to hepatic dissemination through the hepatic portal system. LA was considered of direct extension when it originated from an intra-abdominal source in direct contiguity with the liver. LAs were considered to be cryptogenic in immunocompetent patients when other etiologies could not be determined.

Imaging methods for LA diagnosis were ultrasound, contrast-enhanced computed tomography (CT; liver protocol), and nuclear magnetic resonance. Cultures were performed in anaerobic and aerobic media using standard diagnostic and antibiotic sensitivity tests. The isolated microorganisms were considered responsible for the liver abscess. All patients underwent intravenous antibiotics, initially empirically and later adjusted in accordance to the antibiotic sensitivity tests.

LA drainage was performed percutaneously, guided by ultrasound or CT, or surgically.

2.3. Data Analysis

IBM SPSS Statistics software version 23.0 was used for data analysis. A descriptive analysis was conducted to summarize variables in a manageable form. The data normal distribution was verified using the histogram and the Shapiro-Wilk test. Means, standard deviation, medians, and interquartile range were presented for continuous variables. Absolute and relative frequencies were presented for categorical variables. For group comparison, the chi-square test was used for categorical variables and the Kruskal-Wallis test for continuous variables. Univariate analysis of demographic, clinical and microbiological characteristics was performed. Friedman's test was performed to analyze continuous variables of 3 or more groups. Resistance pattern was defined as a binary variable (without resistant microorganisms / at least one resistant microorganism). For all tests, the p-value of 0.5 (5%) was considered a significant level.

3. Results

Sixty-three patients diagnosed with LA were admitted to the General Surgery Unit between January 2013 and December 2018 (Table 1). Of these, 37 (58.7%) were female. Patients´ age varied between 39 and 97 years, with a mean age of 71.4 years on admission (standard deviation ± 12.37).

3.1. Clinical Characteristics

Abdominal pain and fever were the most common symptoms on admission (73.0% and 61.9%, respectively). No statistically significant differences were noted for specific symptoms between patient groups in relation to the microbiological strain responsible for the infection. However, all patients whose LA was caused by anaerobes had fever on admission (n=6).

A total of 16 (24.4%) patients had diabetes, 1 (1.6%)

was immunocompromised, 8 (12.7%) had concomitant cancer, and 6 (9.6%) had an active history of chronic alcoholism.

Table 1. *Demographic and clinical characteristics of the analyzed population on admission.*

	Study population (n=63)
Demographic characteristics	
Age (years) – mean ± SD	71.4 ± 12.4
Female – n (%)	37 (58.7%)
Clinical characteristics – n (%)	
Abdominal pain	46 (73.0%)
Fever	39 (61.9%)
Vomiting	20 (31.7%)
Shivering	14 (22.2%)
Jaundice	11 (17.5%)
Prostration	8 (12.7%)

SD - Standard Deviation.

3.2. Laboratory and Imaging Data

Leukocytes and C-reactive protein (CRP) were frequently altered analytical parameters on admission. In 92.1% of cases there was an increase in CRP levels above 100mg / L and in 74.6% of cases, leukocytosis was greater than 11.000 x 10^9 / L. In 34.9% of cases, total bilirubin levels were higher than normal. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were high in 39.9% and 46% of patients, respectively.

Median LA diameter was 7 cm (minimum 1.5 cm, maximum 16 cm, and standard deviation ± 3.3 cm). LAs caused by anaerobic agents had a greater median diameter (10.2 cm, p=0.249). In 20 (31.7%) cases, LAs were multiple. Most LAs were located in the right hepatic segments (40 cases, 63.5%). There was bilateral hepatic involvement in 9 cases (14.3%). Table 2 presents laboratory and imaging characteristics of LAs based on isolated microbial agents.

Table 2. *Laboratory and imaging parameters of LA, by isolated microbiological agent.*

	Global (n=63)	Escherichia coli (n=23)	Streptococcus spp. (n=21)	Klebsiella pneumoniae (n=9)	Anaerobes (n=6)	p
PCR (mg/L)	269 [155.1]	184,4 [94.7]	235,6 [59.9]	290,8 [117.1]	365,9 [160.6]	N.S
Leukocytes x $10^{3/L}$	18,5 [8.7]	20 [16.2]	20,2 [5.4]	9,5 [12.2]	20,3 [7.58]	N.S
Bilirubin mg/dL	1,4 [2.25]	1,3 [7.11]	1,2 [1.4]	1,7 [5.35]	1,85 [5.53]	N.S
AST U/L	56,5 [108.5]	45 [102.2]	46 [21]	116 [155]	138 [188.25]	N.S
ALT U/L	60,5 [134]	33 [222]	35 [223]	88 [238.5]	134 [153.5]	N.S
GGT U/L	76,5 [138.8]	125 [264]	125 [284]	161 [178.5]	56 [289]	N.S
ALP U/L	136 [87.8]	167 [219]	153 [210]	113 [125.5]	159 [62.8]	N.S
LHD U/L	624 [322.8]	526 [401]	626 [371]	622 [379.5]	662 [827]	N.S
Diameter[a] (cm)	7 [4.1]	7.8 [3.65]	9 [2]	9 [4.1]	10,15 [5.6]	N.S
Multiple n (%)	20 (27.8%)	5 (21.0%)	9 (42.2%)	5 (55.6%)	1 (16.7%)	N.S
Right liver segments n (%)	40 (55.6%)	18 (78.3%)	14 (66.7%)	4 (44.4%)	3 (57.0%)	N.S
Bilateral n (%)	10 (13.9%)	2 (8.7%)	2 (9.5%)	4 (44.4%)	0	N.S

[a] In case of multiples, the highest is considered.
IQR - Interquartile range; AST- Aspartate amitransferase; ALT - Alanine aminotransferase; GGT - Gamaglutamyltransferase; ALP - Alkaline phosphatase; LHD- Lactic dehydrogenase; N.S. - Non significant.

3.3. Microbiology and Etiology

A total of 79 microorganisms were isolated from LA drainage samples: 41.2% of the samples were polymicrobial and 36.5% had single agents. In 22.2% of the samples, no agents were isolated. The most frequently isolated organisms were *Escherichia coli* (36.5%), *Streptococcus species* (27.8%), *Klebsiella pneumoniae* (11.4%), and anaerobic agents (10.1%).

Blood cultures were performed on admission in 38 patients (58.7%). In 20 (54.1%) cases, microbial agents were not isolated from blood cultures. Of these, 5 had also no agent isolated from the microbiological study of the LA aspirated sample. Of the 18 positive blood cultures, 13 were in accordance with the microbial result of the LA aspirated sample. In one case, no agent was isolated from the LA drainage sample but was isolated from the patient´s blood culture. Of the 79 isolated microorganisms, 10 were multidrug-resistant (MDR), corresponding to 12.6% of the cases. The most frequently isolated bacteria in the MDR group were *Klebsiella pneumoniae* (n=3), *Escherichia coli* (n=3), and *Morganella morganii* (n=2).

Empirically administered antibiotics were carbapenems (57%) and the association piperacillin-tazobactam (38%). Metronidazole was used in combination with another antibiotic in 31.7% of cases. The isolated microbiological agents showed resistance to carbapenems and piperacillin-tazobactam in 26% (n=15) and 18.7% (n=19) of cases, respectively.

With regard to LA etiology, our series showed that 38% of LAs had biliary etiology (n=24), 22% resulted from direct extension of the septic focus (n=14), 10% were of portal origin (n=6), and 30% were cryptogenic (n=19). Hematogenous dissemination cases were not identified. Species of *Streptococcus*, *Escherichia coli,* and *Klebsiella pneumoniae* were the most frequently isolated in all etiological groups, with the exception of portal disseminated abscesses, in which *anaerobes* were the most frequently isolated organisms. *Anaerobes* were not isolated in LA sample cultures of biliary etiology.

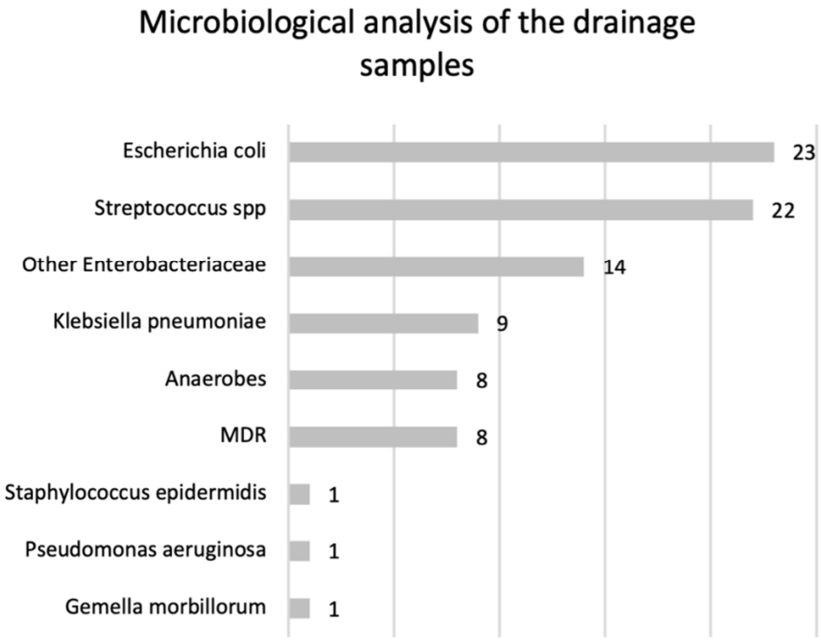

Figure 1. *Microbiological analysis of liver abscess drainage samples.*

Table 3. *Isolated agents by etiological group.*

	Biliary (n=24)	Portal (n=6)	Direct Extension (n=14)	Cryptogenic (n=6)
Streptococcus spp	10	3	3	7
Staphylococcus epidermidis	1	0	0	0
Escherichia coli	10	2	5	6
Klebsiella pneumoniae	4	0	3	2
Other Enterobacteriaceae	10	0	1	1
Anaerobes	0	3	4	1
Pseudomonas aeruginosa	1	0	0	1
Gemella morbillorum	0	0	0	1
Total of isolated agents	36	8	16	19

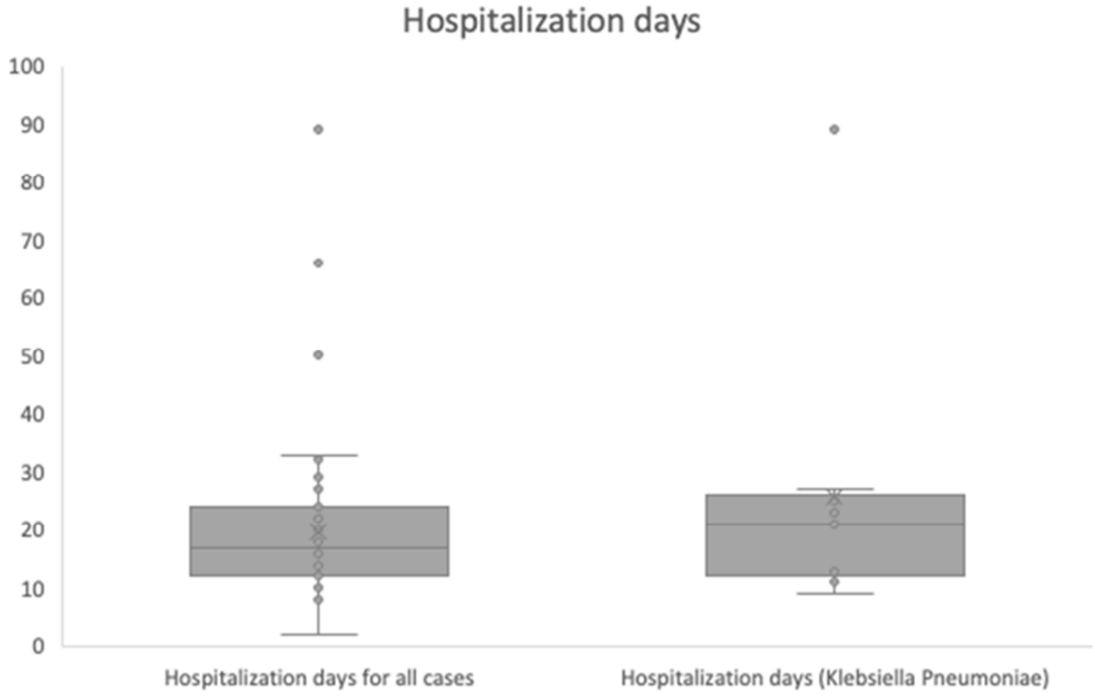

Figure 2. *Hospitalization days for patients with LA (global) and caused by Klebsiella pneumoniae (p=0.07).*

3.4. Treatment

Empiric antibiotic therapy was administered in all cases. In 37 cases (58.7%), a therapeutic adjustment was required after microorganism resistance was found. The median time on intravenous antibiotics was 15 days. In most patients, oral antibiotic therapy was prolonged on an outpatient basis. All patients underwent LA drainage with sample collection for microbiological study. Percutaneous image-guided drainage was performed in 62 (98.4%) cases. Surgical drainage was performed in the remaining 1 case.

3.5. Complications and Outcomes

Median hospital stay was 19.6 days. Patients with LA caused by *Klebsiella pneumoniae* had a higher median hospital stay (25.67 vs 16.50 days, p=0.07) (Figure 2). Rehospitalization was necessary for 11 (17.46%) patients. No major complications were associated with drainage procedures. Four patients had other infectious complications during the hospital stay: 2 patients (3.2%) developed nosocomial pneumonia, 1 (1.6%) endocarditis, and 1 (1.6%) urinary tract infection. In our series, 4 deaths were recorded, corresponding to a 6.3% mortality rate. No statistical difference was found between morbidity and mortality rates relative to isolated microbiological agents.

4. Discussion

In this retrospective study, we carried out a microbiological analysis of patient's LA drainage material and correlated it with clinical, imaging, and laboratory results. Patients´ mean age on diagnosis was 71.4 years which is consistent with other studies [2, 6, 7]. The symptoms at admission were also similar to those described in other studies, in which abdominal pain and fever are the most common symptoms [1, 10, 11].

Laboratory parameters on admission were not pathognomonic or even suggestive of LA diagnosis or of the etiological agent involved. Leukocytosis and increased CRP were the most frequently altered analytical parameters and imaging studies were crucial for LA diagnosis in suspected cases [1]. LA timely diagnosis has direct implications for therapeutic guidance. LA drainage, when indicated, allows for a more effective septic focus control and for the collection of samples for microbiological analysis and antibiogram determination, for further antibiotic selection.

LA microbiological agent evaluation, based on LA etiology (biliary, direct extension, portal, hematogenous or cryptogenic), revealed results consistent with those of the literature [11-13]. Thus, species of the genus *Streptococcus, Escherichia coli,* and *Klebsiella pneumoniae* were the most frequently isolated in all etiological groups, except abscesses of portal etiology, in which *anaerobes* were the most frequently isolated group. This knowledge can help predict the microbiological agents implied in LAs, guiding the choice of the most appropriate empirical antibiotic.

In this regard, it was possible to isolate microbial agents in 77.8% of LA drainage product cultures and 46% of blood cultures. The most frequently isolated agents, namely *Escherichia coli* (36.5%), *Streptococcus* species (27.8%), *Klebsiella pneumoniae* (11.4%), and *anaerobic* agents (10.1%), are consistent with those recorded by other studies [1, 4, 9, 14]. Of the isolated species, 12.5% proved multi-resistant. We identified a higher frequency of LA caused by *Klebsiella pneumoniae* compared to other western series [9, 15]. Our outcomes were similar to those of studies focusing on eastern populations where abscesses caused by this agent have higher associated morbidity [11, 16]. Thus, patients with LA caused by *Klebsiella pneumoniae* had a longer median length of inpatient stay (25.67 vs 16.50 days, p=0.07).

LA mortality is related to sepsis and multiorgan dysfunction [17, 18]. The overall mortality of our series was 6.3% (4 cases), lower than what has been reported in other studies, probably because only LA patients undergoing drainage were included in our study [11, 19, 17]. However, this mortality rate may also reflect improvements in the treatment of these patients in recent years, namely in access to drainage using minimally invasive methods and to more differentiated care units.

Median days of inpatient stay was 17 days, lower than that reported by other studies [4, 9]. These result again, may be biased due to the inclusion in our study, only of patients undergoing LA drainage, but reinforce the fact that and the ideal hospitalization time for these patients is not known or unambiguously defined. Thus, we recommended in most patients, considering patient comorbidities and clinical progression, approximately 2 weeks of intravenous antibiotics followed by 1-4 weeks of antibiogram-guided oral antibiotics.

This study provides a detailed descriptive analysis of LAs in a European Center and an update of the microbiological agents involved, with emphasis on the increasing isolation of MDR bacteria. However, highlighting the limited number of cases of our series is crucial, as well as the retrospective design of the study with its inherent limitations and biases. Never the less our results provide important information for the proper treatment of these patients, with a rare disease without internationally defined guidelines.

5. Conclusions

Despite advances in LA treatment, given its rarity, the high morbidity and mortality rates associated with the disease and the prolonged hospital stays, studies are still needed in order to determine the appropriate therapy for each case. Certain microbiological agents have assumed a predominant role in Asian series, however, few studies explore the most frequently involved microbiological agents in European Centers and their relation with prognosis.

In our series, it was possible to verify that species of the genus Streptococcus, Escherichia coli and Klebsiella pneumoniae are the most frequently isolated, regardless of

the etiological group, with the exception of abscesses of portal etiology, in which anaerobes are the most frequently isolated group. Among the isolated species, 12.5% proved to be multi-resistant and there was also a higher rate of LA caused by Klebsiella pneumoniae, compared to other western series, and that these patients had longer hospital stays.

The results of our series provide important information for the proper therapeutic guidance of patients with this disease, yet without internationally defined guidelines. Despite its retrospective character, this study provides a detailed descriptive analysis of the characteristics of liver abscesses and an update of the microbiological agents involved with emphasis on the increasing isolation of MDR bacteria.

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