

Culture Sensitivity Patterns and Outcome of Liver Abscess in Children Admitted at a Tertiary Care Hospital in North India

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ABSTRACT

BACKGROUND

Liver abscess has been recognised since the time of Hippocrates. Liver abscess is defined as collection of purulent material in liver parenchyma. They are usually caused by bacterial and amoebic infections, and less commonly, by other protozoal and helminthic organisms. Amoebic liver abscess is the commonest extra intestinal site of invasive amoebiasis which mainly affects infants and young children. The incidence of pyogenic liver abscess is much higher among children in developing countries than those in developed countries. The purpose of this study was to evaluate culture sensitivity pattern (Blood & Pus) of liver abscess in children.

METHODS

This prospective observational study was conducted in the Department of Paediatrics, Chacha Nehru Bal Chikistalaya, Delhi from July 2016 –to August 2017. This study has got Institutional Ethics Committee approval (Regd No: IEC/MAMC/78, Dt: 26/07/2016). All children aged 1 month to 12 years admitted with liver abscess (included consecutively) were enrolled after considering inclusion and exclusion criteria. Written and informed consent was taken from parents/guardians of children. Their clinical characteristics, radiological features, laboratory data, clinical management, and outcomes were analysed.

RESULTS

In our study, out of 70 patients, 3.2 % patients showed growth in the blood culture. Organisms isolated were Methicillin resistant *Staphylococcus aureus* (MRSA) 1.4 % (1), *Salmonella typhi* 1.4 % (1), staphylococcus coagulase negative 1.4 % (1). Out of 70 patients of liver abscess enrolled in the study, 36 patients underwent aspiration of pus from the abscess. Out of 36 aspirated cases, gram positive cocci was identified in 1 (1.4 %) patient. In our study, no acid fast bacilli was identified and no fungal culture showed growth of organism. Out of 70 cases of liver abscess, 10 were found to be amoebic liver abscess. In our study, all the 70 patients were started on empirical antibiotics. Out of 70 patients, surgical intervention was done in 36 patients. In our study all the patients were started on empirical antibiotics according to hospital protocol.

CONCLUSIONS

Liver abscess should be considered in children presenting with fever and abdominal pain. Organisms recovered from liver abscesses vary greatly. Surgical drainage has been the traditional mode of treatment of pyogenic liver abscess, but this was replaced by IV broad-spectrum antibiotics and imaging-guided percutaneous drainage.

KEYWORDS

Paediatric Liver Abscess, Amoebic Liver Abscess, Pyogenic Liver Abscess, Culture-Sensitivity, Children

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BACKGROUND

Incidence of liver abscess is more than 79 per 1,00,000 paediatric admissions (< 12 years of age) in India.¹ In paediatric age group, pyogenic liver abscess constitutes to 80 % of the cases and amoebic liver abscess constitute to 10 % of cases.^{2,3} Approximately 40 % of abscesses are mono microbial, an additional 40 % are poly microbial, and 20 % are culture-negative. In pre antibiotic era, culture positivity from pus was less probably due to inadequate culture techniques. Now a days it is further complicated because abscess material is rarely obtained before the administration of antibiotics.⁴ Among cases of pyogenic liver abscesses, staphylococcus is the leading cause in most series.¹⁵ Other bacteria are streptococcus species gram negative rods like *E. coli*,^{5,6} klebsiella,⁷ salmonella and anaerobic organisms (*Bacteroides*, *Fusobacterium*, *Peptostreptococcus*).⁷ *Entamoeba histolytica* or *Toxocara canis*-associated liver abscesses have also been reported in developing countries or in highly endemic areas.⁸ Although many types of bacteria have been identified in liver abscess, there have been several microbiological trends. Organisms recovered from liver abscesses vary greatly but generally reflect bile or enteric flora. Reasons for the variability include differences in antibiotics before culture, cultures techniques or patients' population. *E. histolytica* has been recovered worldwide and is more prevalent in the tropics and subtropics than in cooler climates. The presentations of disease are seen with invasion of the intestinal mucosa and/or dissemination to other organs, the most common being the liver. However, it is estimated that only a small proportion (2 to 8 %) of infected individuals will have invasive disease beyond the lumen of the bowel. After reviewing literature, it has been observed, staphylococcus and *Escherichia coli* are the most common pathogen in children with pyogenic liver abscess.⁹ There is a shift in aetiology to *Klebsiella pneumonia* since 1980. In a study conducted by Liu et al. from 1981 - 1985 first reported that the *Klebsiella pneumonia* was an emerging causative organism of liver abscess.¹⁰ *K. pneumoniae* as the causative pathogen of pyogenic liver abscess occurred in the United States and Europe.¹¹ However, differing from the much higher incidence of *Klebsiella* liver abscess in Asian countries and areas¹² especially the highest incidence in Taiwan that ranges from 80 % to 90 %.¹³ Limited number of studies have been conducted in India regarding aetiopathogenesis of liver abscess in children. In a 6 year retrospective study done by Kumar et al. in Pondicherry, it showed *Staphylococcus aureus* as a common pathogen from pus culture. Other common organism isolated in blood were *E. coli*, *K. pneumonia*, *Salmonella typhi*.¹⁴ In a 5-year retrospective study done by Waghmare et al. in children with liver abscess in west India found that the *Staphylococcus aureus* (9 %) was the most common isolate followed by MRSA, pseudomonas and *Acinetobacter*.¹⁵ Modalities of treatment of liver abscess are conservative (medical management with antibiotics), percutaneous needle aspiration, percutaneous catheter drainage, surgical drainage. For most abscesses, drainage, along with antibiotic therapy is the treatment of choice.

Initial antibiotic therapy should target gram-positive, gram-negative, and anaerobic organisms. Surgical drainage may be necessary in patients with abscess rupture and peritonitis, with large multi-loculated abscesses > 5 cm in size, who do not respond to antibiotics or percutaneous drainage, with concomitant biliary pathology.¹⁶ Surgical drainage has been the traditional mode of treatment of pyogenic liver abscess, but this was replaced by IV broad-spectrum antibiotics and imaging-guided percutaneous drainage either via needle aspiration or percutaneous catheter drainage (PCD). There is a debate about which is better intermittent needle aspiration or PCD. Currently patients with liver abscess are treated with antibiotic along with percutaneous needle aspiration or percutaneous catheter drainage. Surgical drainage is done in patients who fail to respond to antibiotics with PCD drainage.¹⁷

The primary mode of treatment of amoebic liver abscess is medical; however, as many as 15 % of amoebic abscesses may be refractory to medical therapy.¹⁸ Also, secondary bacterial infection may complicate 20 % of amoebic liver abscesses.¹⁹ In such patients and in patients with pyogenic liver abscesses, surgical drainage has been the traditional mode of treatment.²⁰ Change in the mode of treatment may be required because of the dynamic nature of the disease process. Failure to respond to one mode of therapy may call for another mode. Liver abscess can lead to intestinal or extra intestinal complications. Among all pleuropulmonary complications like pleural effusion, empyema, pneumonitis, and hepatopleural fistula are more common.

Other complications are jaundice, ascites, peritonitis, intraperitoneal rupture, rupture into subphrenic space, intrapericardial rupture, pericardial effusion, septic shock. Rare complications like hepatic venous thrombosis and IVC thrombosis can also occur leading to Budd-Chiari syndrome. Outcome of children with liver abscess is measured in terms of morbidity, mortality and duration of hospital stay. There is paucity of data regarding profile of culture (Blood/Pus), antibiotic sensitivity pattern, management, and outcome of liver abscess in Indian children. There is a changing scenario in terms of etiological agent and antibiotic sensitivity pattern.

So we conducted this study regarding the subject which will help us to gain bacteriological profile of liver abscess in northern India and to update antibiotic protocol for management of liver abscess in our institution.

Aims and Objectives

Primary objective

To evaluate culture sensitivity pattern (Blood & Pus) of liver abscess in children.

Secondary Objective

To assess the clinical outcome of patients with conservative treatment, percutaneous needle aspiration and percutaneous catheter drainage in terms of duration of hospital stay, morbidity, and mortality.

METHODS

This prospective observational study was conducted in the Department of Paediatrics, Chacha Nehru Bal Chikistalaya (An Autonomous Institute under Govt. of NCT (National Capital Territory) of Delhi, affiliated to University of Delhi), Delhi from July 2016 to August 2017. This study has got Institutional Ethics Committee approval (Regd No: IEC/MAMC/78, Dt: 26/07/2016). All children aged 1 month to 12 years admitted with liver abscess (included consecutively) enrolled after considering inclusion and exclusion criteria. Written and informed consent was taken from parents/guardians of children. Their clinical characteristics, radiological features, laboratory data, clinical management (Conservative management, antibiotic treatment and surgical interventions), Complications and outcomes were analysed. The outcome of the patient was noted in terms of discharge, death. After the discharge, all patients were followed up for 6 weeks for complete recovery and recurrence of the abscess.

RESULTS

In our study, out of 70 patients, 3.2 % patients showed growth in the blood culture. Organisms isolated were Methicillin resistant staphylococcus aureus 1.4 % (1), *Salmonella typhi* 1.4% (1), staphylococcus coagulase negative 1.4 % (1) (Table-1).

Out of 70 patients of liver abscess enrolled in the study, 36 patients underwent aspiration of pus from the abscess. The aspirated pus was sent for microscopy, grams stain, pus culture (Bacterial, fungal and *Mycobacterium tuberculosis*) and *Entamoeba histolytica* antigen. Direct microscopy was done for identification of *Entamoeba histolytica* trophozoite and trophozoite was identified in none of the patients. Out of 36 aspirated cases, gram positive cocci was identified in 1 (1.4 %) patient. 36 aspirated pus samples were stained with Ziehl Nelsen stain. No acid fast bacilli was identified in all the patients (Table-1).

Pus culture positivity was found to be 3.2 % and 2.9 % patients showed mixed growth. Organisms grown were Methicillin resistant *Staphylococcus aureus* (1), *Staphylococcus hominis* (1), *Streptotrophomonas maltophilia* (1) (Table-1).

All the 36 pus samples were cultured on Sabouraud dextrose agar medium for the growth of fungus. No fungal culture showed growth of organism. All the 36 pus samples were cultured in MGIT for mycobacterium tuberculosis. None of the cultures showed growth of Mycobacterium tuberculosis. Out of 36 cases aspirated, 10 cases (27.7 %) were found to be positive for *Entamoeba histolytica* antigen in pus (Table-1). MRSA was the common isolate in both pus culture and blood culture (Table-1). Out of 70 cases of liver abscess, 10 were found to be amoebic liver abscess. Out of 10 cases ova and cysts of *Entamoeba histolytica* were identified in 5 cases (Table-1). In our study, out of 70 cases enrolled, liquefaction of the abscess was observed in

43 (61.4 %) and remaining 27 (38.6 %) were non-liquefied.

In our study, patients were managed according to the size of the abscess and liquefaction status. Abscess size of < 5 cms was managed conservatively only with antibiotics and 5 – 10 cms were managed with percutaneous needle aspiration along with antibiotics or percutaneous catheter drainage along with antibiotics. Large abscess of > 10 cms size was managed with percutaneous catheter drainage along with antibiotics. In our study, all the patients were started on empirical antibiotics according to hospital protocol. Amoxyclav was started as 1st line antibiotic in 25 cases (35.7 %) (Table-2) and metronidazole was started in all patients. In 45 (64 %) cases, Ceftriaxone and cloxacillin were started as 1st line antibiotics (Table-2). Out of 25 cases started on Amoxyclav, 12 (48 %) cases responded and remaining 13 (52 %) cases were shifted to ceftriaxone and cloxacillin due to clinical deterioration 2nd line antibiotics were meropenem and vancomycin. Out of 70 patients, up gradation of antibiotics was done in 7 patients basing on culture sensitivity report and clinical deterioration (Table-2).

In one case, empirical antibiotics were upgraded to meropenem; in 4 cases, empirical antibiotics were upgraded to vancomycin. 2 patients required up gradation of empirical antibiotics to both meropenem and vancomycin. In remaining 63 cases, no up gradation of antibiotics was required and responded well to 1st line empirical antibiotics (Table-2). In our study, all the 70 patients were started on empirical antibiotics. Surgical intervention was done basing on the size of the abscess and liquefaction. Out of 70 patients, surgical intervention was done in 36 patients (Figure-1).

Out of 36 patients, percutaneous needle aspiration of abscess was done in 15 (41.7 %). Percutaneous catheter drainage was done in 52.8 %. Percutaneous needle aspiration and percutaneous catheter drainage was done in 2 (5.5 %) patients (Figure – 2 & 3).

Out of total 70 patients with liver abscess, 34 were managed conservatively with antibiotics alone, 15 were managed with percutaneous needle aspiration along with antibiotics, 19 were managed with percutaneous catheter drainage along with antibiotics and 2 patients required both PNA and PCD (Figure-3). In our study, out of 70 patients, 38 (54.3 %) patients received parenteral antibiotics for a duration of 1 day -1 week in 1 patient, for a duration of 2 weeks in 38 (54.3 %) patients, for a duration of 3 weeks in 20 (28.6 %) patients, for a duration of 4 weeks in 9 (12.9 %) patients, for a duration of 5 weeks in 2 (2.9 %) (Table - 2). Among 70 cases, 2 patients showed growth of methicillin resistant *Staphylococcus aureus* (1 in blood, 1 in pus). All cultures grown MRSA were sensitive to antibiotics such as vancomycin (100 %), resistant to ciprofloxacin, clindamycin, erythromycin, gentamycin, levofloxacin, oxacillin, penicillin G (Table-2). Among the 70 patients, only one patient showed growth of *Salmonella typhi*. The organism was sensitive to antibiotics such as amoxyclav, cefepime, ceftriaxone, imipenem and meropenem. Intermediate to ciprofloxacin. Resistance was noted to

nalidixic acid, trimethoprim, and sulfamethoxazole (Figure - 4).

Out of 70 patients, 7 patients (10 %) developed pleural effusion and 1 (1 %) patient had rupture of liver abscess.

Remaining 62 patients did not develop any complications (Figure - 5).

In our study, patients were divided into 4 sub-groups based on duration of hospital stay. We found that 14 days of duration of hospital stay in 39 (55.7 %), 15 - 21 days in 20 (28.6 %) cases, 22 - 28 days in 9 (12.9 %) cases, 28 - 42 days in 2 (2.9 %) cases (Figure - 6). Out of 70 patients, 68 were discharged and 2 patients were expired (Table - 2). All 68 discharged patients were followed for 6 weeks after discharge to look for complications and recurrence. None of them were found to have recurrence of abscess or any complications.

Blood Culture Results and Liver Abscess Pus Microscopy / Culture Results / E. Histolytica Antigen Tests / Stool for Ova and Cysts Results.			
		Frequency (no)	Percentage (%)
Growth in blood culture	MRSA	1	1.4
	Salmonella typhi	1	1.4
	Coagulase neagtive staphylococci	1	1.4
	No growth	67	95.7
	Total	70	100
Gram stain	Positive	1	1.4
	Negative	35	50
	Not aspirated	34	48.6
	Total	70	100
Growth in pus culture	MRSA	1	2.8
	Staphylococcus hominis	1	2.8
	Stenotrophomonas maltophilia	1	2.8
	Mixed	2	5.5
	Growth absent	31	86
	Total	36	100
Entamoeba histolytica antigen	Positive	10	14.3
	Negative	26	37.1
	Not aspirated	34	48.6
	Total	70	100
Stool for ova and cyst of Entamoeba histolytica	Present	5	7.1
	Absent	65	92.9
	Total	70	100

Table 1. Distribution of Culture (blood/pus) Results, Microscopy, Antigen Testing and Stool Tests Results in the Present Study Population

Antibiotic Susceptibility Patterns, Duration of Treatment and Outcome			
		Frequency (Number)	Percentage (%)
Amoxy-CLAV	Yes	25	35.7
	No	45	64.3
	Total	70	100
Ceftriaxone & cloxacillin	Yes	58	82.9
	No	12	17.1
	Total	70	100
Antibiotic upgradation	Meropenam	1	1.4
	Vancomycin	4	5.7
	Meropenam and Vancomycin	2	2.9
	No upgradation	63	90
	Total	70	100
Vancomycin sensitivity patterns	Sensitive	2	100
	Resistant	0	0
	Total	2	100
Clindamycin/Ciprofloxacin/Gentamycin/Levofloxacin/Oxacillin/Penicillin G	Sensitive	0	0
	Resistance	2	100
	Total	2	100
Durations of antibiotic treatment	1 day - 1 week	1	1.4
	2 weeks	38	54.3
	3 weeks	20	28.6
	4 weeks	9	12.9
	5 weeks	2	2.9
	Total	70	100
OUTCOME	Discharge	68	97.1
	Death	2	2.9
	Total	70	100

Table 2. Distribution of Medical Management (Antibiotics) Details in the Present Study Population

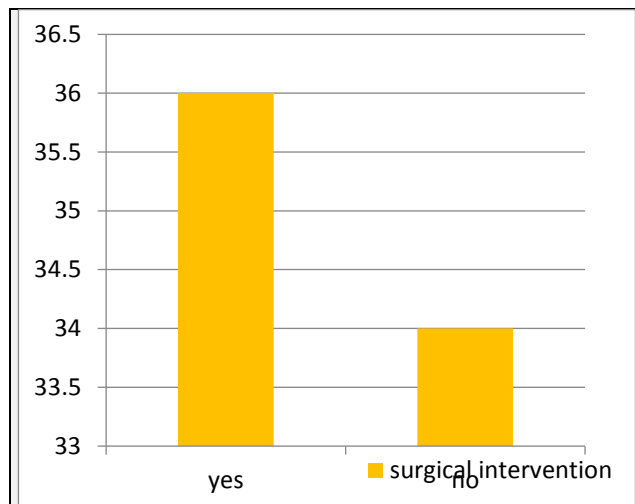


Figure 1. Distribution of Surgical Intervention (Yes/No) in the Present Study Population

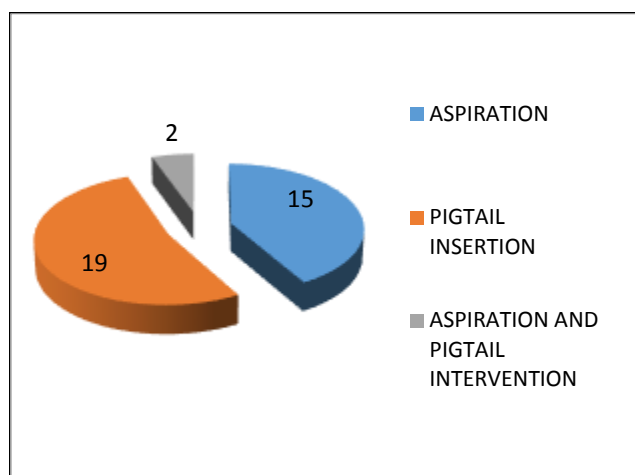


Figure 2. Distribution of Different Surgical Interventions among the Present Study Population

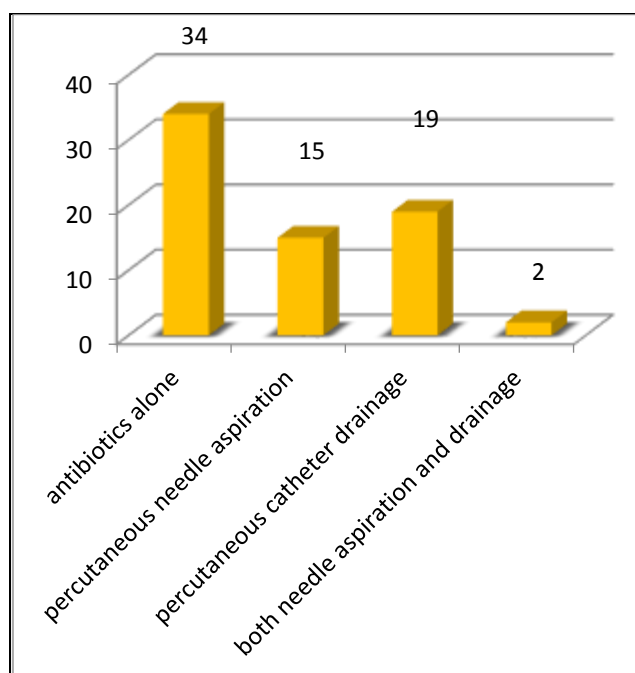


Figure 3. Distribution of Management Modalities among the Present Study Population

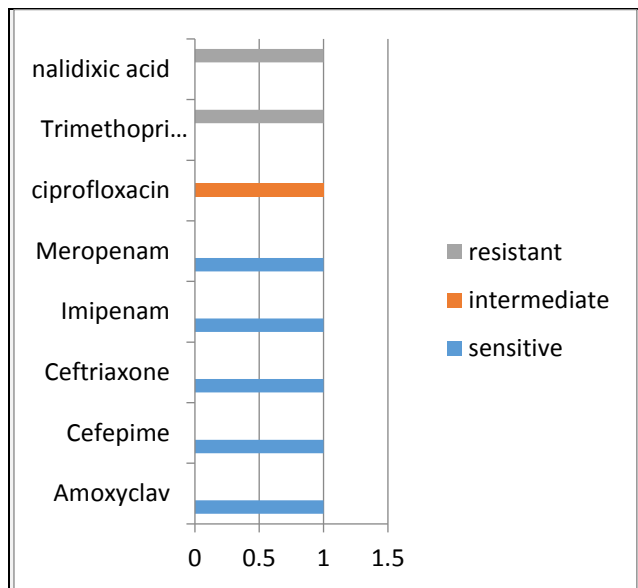


Figure 4. Distribution of Antibiotic Sensitivity Patterns *Salmonella typhi* Isolated in the Present Study Population

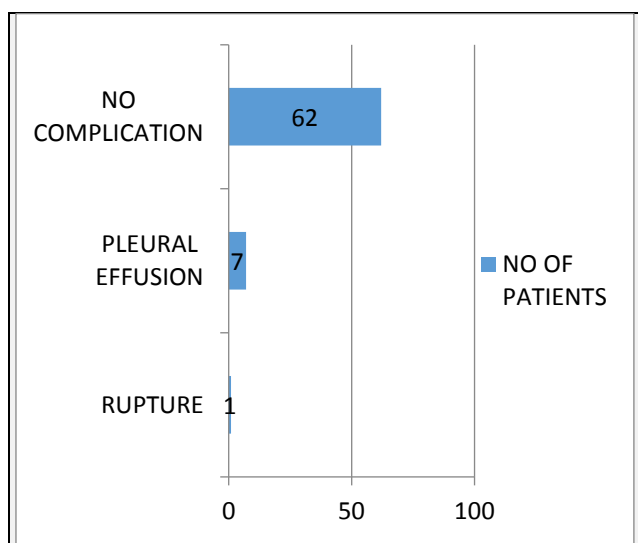


Figure 5. Distribution of Complications among the Present Study Population

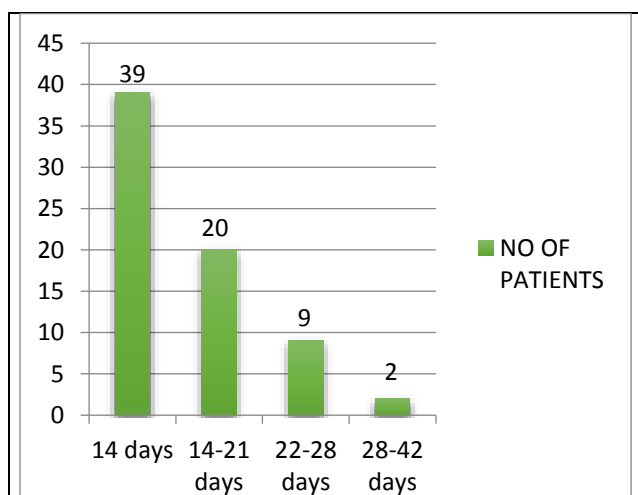


Figure 6. Distribution of Duration of Hospital Stay among Present Study Population

DISCUSSION

Organisms isolated from liver abscess patients vary widely. In our study, out of 70 patients, aspiration of the abscess was done in 36 patients and pus culture positivity was 8 % (3). Blood culture was done in all patients of liver abscess and positivity was 4 % (3). The most common etiological agent was Methicillin resistant *Staphylococcus aureus* (MRSA) which was isolated from both pus (2.8 %) and blood culture (1.4 %). Other etiological agents isolated were *Salmonella typhi* (1.4 % in blood culture), *Stenotrophomonas maltophilia* (2.8 % in pus culture), *Staphylococcus hominis* (2.8 % in pus culture), coagulase negative staphylococci (1.4 % blood culture). Salahi et al. in his study found that *Staphylococcus aureus* (62 %) was the most common organism causing liver abscess.²¹ Ferreria et al. also found that the *Staphylococcus aureus* was the most common isolate from pus of the patients with liver abscess.²² Few other studies done in children shows different results with the growth of *Escherichia coli* and *Klebsiella* in aspirated pus samples.²³ All the 36 pus samples were subjected to entamoeba antigen detection tests and total 10 samples were positive for *Entamoeba histolytica* antigen (27 %). Haque et al. in a study done in Virginia found that out of 27 liver abscess pus specimens, 11 (41 %) were positive for lectinantigen.²⁴

In our study, *Entamoeba histolytica* was detected by antigen tests. Anaerobic cultures were not performed due to non-availability of kit in our institution. It can be noted that parasitic etiological agent is a causative agent of liver abscess in paediatric group beside bacteriological agent. *Stenotrophomonas maltophilia* was a rare organism that was isolated in 1 of our patients. According to evidence available, it is considered that a positive blood culture growth for *Stenotrophomonas* is usually due to colonisation in indwelling catheter. But in our case this organism was grown in pus culture which is significant. In our study, the low level of positivity in blood culture and pus culture could partly be due to prior antibiotic therapy before admission. In our study, it has been noted that *Entamoeba histolytica* antigen was detected in highest number of cases (27 %) but growth of *Entamoeba histolytica* and demonstration of trophozoites in pus is gold standard in diagnosis of amoebic liver abscess. In our study, none of the pus samples showed trophozoites in microscopy and anaerobic culture was not done due to non-availability of facilities in our institution. So it can be stated that *Entamoeba histolytica* is a parasitic agent in causation of liver abscess but not the commonest organism. In our study, ultrasonographically we confirmed size, volume, liquefaction status of the liver abscess to decide the management plan as per the protocol. While observing the volume of the abscess, we found highest number of patients (38.6 %) had abscess of volume less than 50 ml followed by abscess of 50 ml – 100 ml volume in 30 %, abscess of 100 – 200 ml volume in 17.1 %, and abscess of >200 ml volume in 14.3 %. In our study, most of the patients (61.4 %) had abscess with liquefaction. In our study, patients with abscess size of < 5cms were managed conservatively with antibiotics, abscess size of 5 – 10 cms

managed with aspiration only or percutaneous catheter drainage in patients requiring repeated aspiration along with antibiotic therapy. Patients with large abscess > 10 cms underwent percutaneous catheter drainage along with antibiotic therapy. None of the patients underwent open surgical drainage (laparotomy). In patients requiring surgical intervention, 41.7 % patients were managed with percutaneous needle aspiration and percutaneous catheter drainage was done in 52.8 %. Two patients required both percutaneous needle aspiration and percutaneous catheter drainage after repeated aspirations (5.5 %). Ghosh et al. in his study managed the liver abscess patients with conservative treatment in < 5 cms abscess size group and conservative treatment followed by percutaneous drainage were done in patients with abscess of > 5 cms size not responding to antibiotic therapy.²⁵ In a study done by Amin et al. in Gujarat managed patients with abscess size of less than 5 cms with antibiotics alone, patients with abscess size of 5 cms – 10 cms with antibiotics and percutaneous needle aspiration and patients with abscess size of more than 10 cms with antibiotics and percutaneous catheter drainage.²⁶ Mangukiyaya et al. in his study reported that percutaneous needle aspiration was the safe and effective treatment and 56 % responded well.²⁷ Out of 70 patients, 34 (48.6 %) patients were managed conservatively alone with the empirical antibiotics amoxicillin/ceftriaxone and cloxacillin with metronidazole. Remaining 36 (51.4 %) patients underwent surgical intervention along with antibiotic therapy as per management protocol. All patients were started with empirical antibiotic therapy as per institutional protocol. In 25 patients empirical antibiotic therapy was started with intravenous amoxiclav as a 1st line of antibiotic. But 13 patients had to be shifted to ceftriaxone and cloxacillin due to clinical nonresponse. Total number of 45 patients had received ceftriaxone and cloxacillin as a 1st line of antibiotics. In ceftriaxone and cloxacillin group, antibiotic had to be upgraded to IV meropenem and IV vancomycin in 7 patients. Antibiotic were changed according to culture and sensitivity report in culture positive patients. All patients received metronidazole for initial 10 days empirically. In patients who had positive antigen test for *Entamoeba histolytica*, empirical antibiotics were continued for total duration of 6 weeks suspecting super added bacterial infection. Parenteral antibiotics were shifted to oral antibiotics on the basis of duration (minimum of 2 weeks IV), afebrile period for more than 72 hours. Total duration of antibiotic therapy was 6 weeks irrespective of culture positivity status. In a study done by Shrivastava et al. total duration of antibiotic given in study group was 6 weeks.²⁰ In our study, 7 patients had pleural effusion and 2 patients had empyema and managed accordingly as per protocol. One patient had rupture of liver abscess. Mean duration of hospital stay in our study was 14 days +/- 6 days. Singh et al. in a study found that the mean duration of hospital stay in liver abscess patients was 22 days.²⁸ In a study done by Yacaria et al. in paediatric patients with liver abscess the mean duration of hospital stay was 13 days.²⁹ Mortality in our study was 3 %. One patient expired due to rupture of liver abscess and another patient expired due to septic shock.

With improvement of diagnostic modality, early surgical intervention and antibiotic therapy has brought down the mortality due to liver abscess from as high as 36 % reported by Donovan et al.³⁰ in 1998 to 5.5 % reported by Salahi et al.³¹ in 2007. However, studies done by Muroh et al. and Hsu et al. reported that no mortality was seen in patients with liver abscess in their study.³² All the patients were followed for a total duration of 6 weeks after discharge. No patients had any recurrence of abscess during follow up.

CONCLUSIONS

Liver abscess should be considered in children presenting with fever and abdominal pain. Commonest bacterial pathogen causing liver abscess was methicillin resistant *Staphylococcus aureus* followed by *Salmonella typhi*, *Stenotrophomonas maltophilia*, coagulase negative *Staphylococcus aureus* and *Staphylococcus hominis*. *Entamoeba histolytica* is a common parasitic agent causing liver abscess in children. Small sized abscess (size of less than 5cms) was managed conservatively. Majority of patients required antibiotic therapy and surgical drainage. Average duration of IV antibiotics was for 2 weeks. Organisms grown in the culture were sensitive to commonly used antibiotics like ceftriaxone and vancomycin. Common complication noted among patients with liver abscess was pleural effusion. Mortality rate was low as 3 %. All the patients were healthy and none of them had recurrence of abscess during the follow up. Pus cultures, blood cultures should be sent before starting antibiotics for better yield of cultures of organisms. From our study, we recommend ceftriaxone, cloxacillin and metronidazole as the first line antibiotics in liver abscess patients. In our study, anaerobic culture for pus and blood could not be done due to lack of facilities. Due to this, growth of *Entamoeba histolytica* and other anaerobic organisms could not be isolated. However, in our study we found high positivity of entamoeba antigen in pus.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.

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