

## Original Research Article

## Microbiological flora, demography and presentation of liver abscess: A changing trend

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**Abstract**

**Objective:** A prospective study in Rohilkhand region to analyse the causative organism for liver abscesses which might be helpful in planning the management of liver abscesses. **Methods:** Patients who were diagnosed to have liver abscess of size 5 cm or more and aspiration of pus was done either by percutaneous or by pig tail insertion were included in the study. The study includes all the patients who came in OPD or admitted in the hospital. The study was conducted for a period of one year. **Results:** The prevalence of liver abscess shows male predominance. Age dependency with significantly higher prevalence in the age group of 35 to 55 years. Right upper quadrant pain, fever and anorexia was seen in 64 (91%), 55 (78 %) and 50 (71 %) cases respectively. Patients with amoebic liver abscesses were more associated with diarrhea and cough while fever, pain in right upper abdomen and nausea or vomiting was more associated with pyogenic liver abscesses. The most common organism cultured was *Klebsiella pneumoniae* (23.3%) followed by *Escherichia coli* (13.3%). The detection of *E. histolytica* in aspirated pus was 70 % while only 10 % in stool samples. **Conclusion:** Pyogenic liver abscess can be monomicrobial or polymicrobial and may be associated with amoebic liver abscess. Abscesses, which were previously thought to be "sterile" or cryptogenic, may in fact be caused by anaerobic organisms that were not previously identified secondary to inadequate technique. Compared to pyogenic liver abscesses, patients with amoebic abscesses are often younger, more acutely ill and are usually from high prevalence areas. The sensitivity of PCR was higher as compared with microscopy in this study in detecting *E. histolytica*.

**Keywords:** Pyogenic Liver Abscess, Monomicrobial, Polymicrobial, Amoebic Liver Abscess.

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**Introduction**

Liver abscesses are common hepatic infections in India. They are mostly amoebic or pyogenic. In India amoebic liver abscess are more common than pyogenic abscess due to poor socioeconomic status, consumption of untreated contaminated water, lack of urban amenities and habit of alcohol consumption. Amebiasis, caused by *Entamoeba histolytica* is common in Indian population and is a significant cause of morbidity and mortality especially if patient develops amoebic liver abscess.

Pyogenic liver abscesses are often related to specific pathogens. With the advent of radiological and culture facilities, organisms can be easily cultured and identified by direct aspiration of pus from the abscess cavity. It has been shown in various studies that culture from the aspirate has higher positive culture rates than those of blood culture samples, and only in 50% of patients with pyogenic liver abscesses have both cultures positive[1]. Negative culture reports from aspirated material is although high and it has been reported in about 20% of patients[2]. The reason for negative culture reports might be due to poor culture technique, delay in plating due to unavailability of culture facility or improper technique of transport of the aspirated fluid. Negative cultures can also be caused by the use of broad-spectrum antibiotics before cultures are obtained. Moreover it has been shown in various studies that negative culture reports are more common in cryptogenic liver abscess. Most of the liver abscesses secondary biliary tract disorders are more likely to have positive cultures from both blood and aspirated pus[3].

In Asian populations, *Klebsiella pneumoniae* is the most frequent pathogen associated with pyogenic liver abscesses especially in Taiwan while *E. coli* is the most common pathogen in Western countries, in both monomicrobial and polymicrobial isolates, followed by *Streptococcus milleri*. Anaerobes may also be cultured from pyogenic liver abscesses and *Bacteroides* spp. were most commonly isolated[4]. As there are quite variation in the microbiological flora of liver abscess, we therefore conducted a prospective study in Rohilkhand region to analyse the causative organism for liver abscesses which might be helpful in planning the management of liver abscesses.

**Materials and methods****Study design/Type of study**

This was a prospective study conducted at Rohilkhand medical college and hospital, Bareilly, U.P., India

**Sample size & Duration of study**

All the patients who were diagnosed to have liver abscess of size 5 cm or more and the aspiration of pus was done either by percutaneous or by pig tail insertion were included in the study. The study includes all the patients who came in opd or admitted in the hospital. The study was conducted for a period of one year.

**Inclusion & Exclusion criteria**

All the patients who were admitted in the hospital with diagnosis of liver abscess clinically and radiologically either by ultrasonography (USG) and/ or CT scan with size more than 5 cm were included in this study. Exclusion Criteria were age less than 18 years, pregnant women, abscesses that required transpleural drainage and ruptured abscesses.

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**Data collection procedure**

Written consent, blood investigations including coagulation profile, and correction of coagulopathy were performed prior to the procedure. Percutaneous aspiration or percutaneous catheter drainage of liver abscess were done under ultrasound guidance. Percutaneous drainage were performed using self-locking pig-tail catheters of size range 10 or 12 French under USG guidance. Aspiration of the abscess material was then performed until no more pus could be aspirated. Aspirate was sent for culture and sensitivity. All the samples were brought to the laboratory immediately after collection and examined macroscopically and microscopically.

**Observation chart****Patient demography**

A total of 70 cases of liver abscess from 59 (84.2%) males and 11 (15.7%) females were included and studied. Ten cases were excluded, as we were not able to aspirate the pus as abscess cavity was having organized thick non-aspirable pus.

**Clinical characteristics of liver abscess infections**

Symptoms	Amoebic (40)	Pyogenic (30)
Anorexia	30	20
Right upper quadrant pain	36	28
Hepatomegaly	28	20
Fever	25	30
Nausea/vomiting	05	18
Cough	10	05
Diarrhea	06	02
Right pleural effusion	06	03

The overall prevalence of liver abscess showed age dependency with significantly higher prevalence in the age group of 35 to 55 years. The mean age of participants was found to be  $40 \pm 12.3$  years. Right upper quadrant pain, fever and anorexia was seen in 64 (91%), 55 (78 %) and 50 (71 %) cases respectively. Patients

**Culture characteristics of liver aspirate**

Liver abscess	Culture result	Microbiology	Number	Percentage
Pyogenic liver abscess	No growth	No growth	15	50
	Culture positive	Klebsiella	07	23.3
		E. coli	04	13.3
		Pseudomonas	02	6.6
		S. aureus	02	6.6
Amoebic liver abscess		Entamoeba Histolytica	28	70

**Results**

A total of 70 cases of liver abscess from 59 (84.2%) males and 11 (15.7%) females were included and studied. The overall prevalence of liver abscess showed age dependency with significantly higher prevalence in the age group of 35 to 55 years. The mean age of participants was found to be  $40 \pm 12.3$  years. Right upper quadrant pain, fever and anorexia was seen in 64 (91%), 55 (78 %) and 50 (71 %) cases respectively. Patients with amoebic liver abscesses were more associated with diarrhea and cough while fever, pain in right upper abdomen and nausea or vomiting was more associated with pyogenic liver abscesses.

In patients with pyogenic liver abscess, 50 % of the culture from the aspirate shows no growth. The most common organism cultured was *Klebsiella pneumoniae* (23.3%) followed by *Escherichia coli* (13.3%), *Pseudomonas* in 6.6 % and *Staphylococcus aureus* in 6.6 % of the cases. Entamoeba was detected in 70 % cases with anchovy sauce aspirate. Out of 40 suspected amoebic liver abscess, only 04 (10%) liver aspirate samples were found to have for *E. histolytica* trophozoites on microscopy but when these samples were sent for PCR, 70% (28/40) were found to be positive for *E. histolytica*. No other species of Entamoeba genus was found in any of the liver aspirates. Stool samples were analysed in about 20 of which 2 were found to be positive for Entamoeba cysts/ trophozoites through microscopy. The sensitivity of PCR was higher as compared

**Microscopy**

Direct wet mount microscopy was performed for all the samples with anchovy sauce aspirate to screen for the presence of trophozoites only and trophozoites and cysts of Entamoeba in pus and stool samples respectively. PCR was done in patients with anchovy sauce aspirate in which direct wet mount microscopy was negative.

**Aerobic culture of liver aspirate**

All the abscess aspirates were inoculated on Blood agar media and Mac Conkey agar and incubated at  $37^{\circ}\text{C}$  for overnight. Further growth on the culture plates were identified by colony morphology, Gram staining and standard biochemical tests.

with amoebic liver abscesses were more associated with diarrhea and cough while fever, pain in right upper abdomen and nausea or vomiting was more associated with pyogenic liver abscesses.

with microscopy in this study. The detection of *E. histolytica* in aspirated pus was 70 % while only 10 % in stool samples.

**Statistical analysis**

Data was compiled using MS excel 2007 and analysis was done with the help of Epi-Info 7 software. Frequency and percentage were calculated & statistical test (Chi Square) was applied wherever applicable;  $p<0.05$  was taken as statistically significant.

**Discussion**

Liver abscess is a disease known to us from past 100 years. Demographics, etiology, diagnosis and treatment has been changed drastically with improving socio economic status, better hygiene, improving radiological and culture facilities. Traditionally it has been classified into two broad categories: those of bacterial origin, otherwise known as pyogenic liver abscess and those of parasitic origin primarily caused by *Entamoeba histolytica* leading to amoebic liver abscess. Country like India, liver abscesses are still a commonly encountered liver disease leading to significant morbidity and mortality. The incidence of pyogenic liver abscess is increasing as prior to 1970 the incidence was 5–13 patients per 100,000 admissions but in the present decade had been increased to approximately 22 cases per 100,000 admissions[3].

Pyogenic liver abscesses are mostly associated with secondary spread of infective foci involving the other organs most commonly enteric or biliary system. There have been many series in the literature, which have identified multiple species of bacterial flora presumed to be etiologic agents responsible for hepatic abscesses. *Escherichia coli*, *Streptococcus*, *Enterococcus*, and *Klebsiella* are often cultured in patients with pyogenic liver abscesses, with *E. coli* and *Streptococcus* being the most frequently isolated bacterial flora in most series in the Western literature[8,9].

There appears to be some geographic correlation with culture isolates in patients with pyogenic liver abscesses, as *Klebsiella pneumoniae* is especially prevalent in Asia when compared to Western populations[10-12]. However, there are many confounding factors that make it difficult to generalize regarding the etiology of pyogenic liver abscess. Most patients receive antibiotic therapy prior to obtaining liver abscess or blood culture, which may make it difficult to isolate the offending pathogen. Evolution and refinement of culture technique have led to the increasing identification of anaerobic and microaerophilic organisms in pyogenic liver abscesses. *Bacteroides* species, anaerobic *Streptococcus*, and *Fusobacterium* species are the most common anaerobes[7,13].

Singh et al in their study found that among the 115 samples, 50 (43.4%) showed the presence of the anaerobe. The most common anaerobes was *Fusobacterium* (19, 27.9%) and *Peptococcus* (19, 27.9%), followed by *Prevotella* (18, 26.4%), *Bacteroides* (8, 11.7%), *Peptostreptococcus* (2, 2.9%) and *Clostridium* (2, 2.9%). In context of anaerobic bacterial flora, majority (74%) of the samples were monomicrobial. In relation to amoebic etiology mixed with bacterial superimposed infections.

*Prevotella* and *Fusobacterium* were found to be most commonly associated with the *E. histolytica*[5, 6]. The improvement in culture technique, especially anaerobic techniques, suggests that abscesses, which were previously thought to be "sterile" or cryptogenic, may in fact be caused by anaerobic organisms that were not previously identified secondary to inadequate technique.

Pyogenic liver abscesses due to hematogenous spread not associated with a gastrointestinal source are often monomicrobial, the result of infection with *Staphylococcus aureus* or *Streptococcus* species and tends to form solitary abscesses. In contrast, infections that are from enteric or biliary sources tend to be polymicrobial and associated with aerobic gram-negative bacteria and anaerobes[14-16]. The predictions for the presence of microflora could not be made based on the clinical characteristics of the abscess<sup>7</sup>. Before the 1980s, *E. coli* was the most common pathogen that caused pyogenic liver abscesses and was mostly polymicrobial. However, during the past two decades, highly virulent strains of *K. pneumoniae* had emerged as a predominant cause of pyogenic liver abscesses in Asian countries, United States and Europe[8-12,17].

Ghosh et al in their study of etiological analysis of liver abscess revealed that 69% were of amoebic origin ( $n = 138$ ), 18% of pyogenic ( $n = 36$ ), 4% of mixed amoebic and pyogenic process ( $n=8$ ), 7.5% of tubercular ( $n = 15$ ), and 1.5% of fungal infections ( $n=3$ ). Pus culture gave positive results in 22% ( $n = 44$ ) of the patients, which grew predominantly Gram negative flora. Amoebic liver abscesses tend to affect younger population especially males. Common presenting complaints were abdominal pain, fever, and weight loss. Etiologically, Gram negative organisms commonly inhabiting the gut and biliary microflora were frequently encountered by them, *E. coli* being the most common pathogen followed by *Klebsiella*, *Pseudomonas* and *Staphylococcus*[15].

Serraino et al done their study in Italy over 109 patients of pyogenic liver abscess. Most common symptom was fever (73%) in their study followed by right upper abdominal pain in 63.3%, vomiting and nausea in 28.4%. Out of 99 cases on culture, only 53.5% of the cases came with positive microbial reports<sup>18</sup>. Culture from aspirate of liver abscess was positive in 25 of 62 patients who underwent percutaneous aspiration of liver abscess (positive rate, 40.3%). The most common organism identified was *E. coli* (26.5%), followed by *Streptococcus* spp (13.2%) and anaerobics (13.2%). Other

organisms were *Enterococcus* spp (11.3%), *Staphylococcus* (7.5%) They observed *E. coli* infections as a predominant organism identified on culture reports followed by *Streptococcus* spp, *Enterococcus* spp and anaerobics. In only 2 cases *K. pneumoniae* were identified which is the most common infection seen in patients with pyogenic liver abscesses in Southeast Asia[19]. The high incident of *E. coli* and *Enterococcus* spp is probably related to the high incidence of biliary cause of pyogenic liver abscesses[20].

Wang JH, Liu YC et al in their study over primary liver abscess due to *Klebsiella pneumoniae* in Taiwan reviewed 182 cases of pyogenic liver abscess during 6 years; 160 of these cases were caused by *K. pneumoniae* alone, and 22 were polymicrobial. Liver abscess caused by *K. pneumoniae* is a new clinical syndrome that has emerged as an important infectious complication in diabetic patients in Taiwan[21]. Compared to pyogenic liver abscesses, many studies have reported that patients with amoebic abscesses are often younger, more acutely ill with fever and right upper quadrant pain, and are usually from high prevalence areas[22,23]. The mean age of our patients with amoebic liver abscess was 40 years and was comparable to other studies<sup>22</sup>. The frequency of fever and pain abdomen was 67-87% and 62-94% of patients with amoebic liver abscess respectively in different series [24]. In our study, most common symptoms were right upper abdominal pain in 90% and anorexia in 75 % of the cases. PCR was positive in 70% of pus specimens from patients with amoebic liver abscesses while microscopy was positive only in 10 % of the cases. This has been shown in many studies that PCR is better than microscopy in detection of Entamoeba. In study done by Singh et al over prevalence of amoebic liver abscesses also observed the similar results with detection rate of 87.8 % with PCR as compared to 5.2 % by microscopy. They concluded that the high sensitivity of PCR in their study may be due to use of nested PCR for the detection[25]. The sensitivity of PCR is also high in identifying the species as compared to microscopy[26,27] as *E. histolytica* is morphologically similar to *E. dispar* and *E. moshkovskii* and thus difficult to differentiate using microscopy.

For higher detection of trophozoites by microscopy, the analysis of aspirate should be done as soon as possible as trophozoites remain motile or viable for only a few minutes after coming in contact with air after aspiration and thus makes the detection even difficult by microscopy. The chances of detection on microscopy also depends on the concentration of trophozoites in aspirate. The concentration of trophozoites are more if collection of aspirates done at the end of the percutaneous needle aspiration or pig tail insertion. Stool examination for the diagnosis of Entamoeba Histolytica is also not sensitive in patient with amoebic liver abscesses. In our study although stool examination was done only in 20 cases of liver abscesses but *E. histolytica* was detected only in 2 patients on stool examination. In our study, we observed that *Klebsiella Pneumonia* was the most common organism isolated on culture from liver aspirate while in amoebic liver abscess, PCR was more sensitive in diagnosing as compared to microscopy in patients with liver abscess of size 5 cm or more in longest diameter.

### Conclusion

Pyogenic liver abscess can be monomicrobial or polymicrobial and may be associated with amoebic liver abscess. Abscesses, which were previously thought to be "sterile" or cryptogenic, may in fact be caused by anaerobic organisms that were not previously identified secondary to inadequate technique. Compared to pyogenic liver abscesses, patients with amoebic liver abscesses are often younger and are usually from high prevalence areas.

### What this study add to existing knowledge

With the advent of Culture techniques and availability of the experts and laboratories in sub urban areas in last two decades lead to increased positive reports on culture of aspirate from the abscess cavity using percutaneous techniques in aseptic environment. Aspirate from abscess cavity is more likely to give positive isolate on culture as compared to blood culture. Pyogenic liver abscess can be

monomicrobial or polymicrobial and may be associated with amoebic liver abscess. About 48 to 55% of hepatic abscess cultures were being polymicrobial in origin.

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