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Bacterial Profile and Antibiogram of Biliary Pathogens in Patients with Hepatolithiasis

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Abstract **Original Research Article**

***Background*:** Hepatolithiasis is usually associated with contaminated bile. In hepatolithiasis, frequently presence of stones and stricture within the intrahepatic bile duct that causes stasis of bile. This bile frequently contaminated with various types of bacteria. So culture and sensitivirty test can find out the nature of bacteria and its antibiogram that can be used as empirical therapy for cholangitis in hepatolithiasis. ***Materials and Methods*:** This study was conducted at the Department of Hepatobiliary, Pancreatic & Liver Transplant Surgery of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, with 31 patients with hepatolithiasis who underwent surgical procedure from December 2019 to November 2020 with purposively sampled hepatolithiasis patient. Bile was collected intra- operatively from the bile duct for culture and antibiotic sensitivity. ***Results*:** The median age was 38 years (range 25-80 years). There were 19 males and 12 females. Abdominal pain was present in all patients with hepatolithiasis. Out of 31 patients, 24 patients had positive bile culture; E. coli was the most frequent organism (n=13, 41.9%) followed by Klebsiella spp. Colistin, imipenem and Tigecycline (100%), doxycycline (83.3%),amikacin (82.6%), Meropenem & Tazobactam+ Piperacillin (72.7%) and cefepime (66.7%) were sensitive to these organisms. ***Conclusion*:** Cephalosporins and Fluoroquinolones are not good antibiotics using empirically as prophylactic measure for patients with hepatolithiasis in our setting. Imipenem, meropenem, doxycycline, amikacin, cefepime and Tazobactam/piperacillin can be used empirically in hepatolithiasis patients. However further study with larger sample size is recommended.

Keywords: Hepatolithiasis, choledocholithotomy, laparoscopic.

INTRODUCTION

Hepatolithiasis is defined as presence of gallstones proximal to confluence of right and left hepatic duct with or without gallstones in the gallbladder and/or common bile duct [7]. Hepatolithiasis are frequently found in south-east Asia. China, Hong Kong, Taiwan, Korea, and Japan are the countries mostly found hepatolithiasis and the incidence rates ranging from 2% to 25% [5]. Hepatolithiasis is found 0.6%-1.3% of patients in western countries [6]. The mechanism of hepatolithiasis is unknown but some factors can contribute stone formation within the intra hepatic bile duct such as malnutrition, poor hygiene and low socioeconomic status [5]. Gallstones have large amounts of calcium bilirubinate and remain in the intra- and/or extrahepatic bile ducts, and the presence with bacteria [11]. In hepatolithiasis due to presence of stones in the intrahepatic bile duct that causes stasis of the bile. However, stricture of the bile duct causes

further stasis of the bile. Stasis of the bile evokes bacterial contamination and bacteria overgrowth in the bile within the bile duct of the liver. This contaminated bile frequently cuases cholangitis and further stricture in the bile duct. Stones within the intrahepatic bile duct remain prolong periods that leads to recurrent cholangitis, intrahepatic biliary strictures, hepatic parenchymal atrophy, secondary biliary cirrhosis and cholangiocarcinoma [2]. This study was undertaken to identify common bacteria in bile of hepatolithiasis patient and to determine antibiotic sensitivity which can be used as prophylactically in treating cholangitis.

OBJECTIVE OF THE STUDY

General Objective

To determine the bacterial profile and antibiogram of biliary pathogens in patients with hepatolithiasis.

Specific Objectives

1. To identify the microorganisms in bile of patients with hepatolithiasis.
2. To understand the antibiotic sensitivity of biliary pathogens.
3. To identify the apprppriate antibiotic for prophylactic use in cholangitis.

METHODOLOGY

Study Design

Cross sectional study.

Place of Study

Department of Hepatobiliary Pancreatic & Liver Transplant Surgery, Bangabandhu Sheikh Mujib Medical University.

Study Period

One year (December 2019-November 2020).

Study Population

All patients of either sex who underwent biliary surgery in presence of hepatolithiasis during the study period in the Department of Hepatobiliary Pancreatic & Liver Transplant Surgery, Bangabandhu Sheikh Mujib Medical University.

Sample Size

In our study period total 31 samples were collected.

Enrollment Criteria

Inclusion Criteria

1. Patient who underwent surgery for hepatolithiasis.

Exclusion Criteria

1. Patient unwilling to give consent.
2. Patients having multiple comorbidity.

Data Collection

Prior to data collection, both verbal and written consent had been taken from the patients and the investigator himself collected data. Data was collected on variables of interest. All the investigations were done in Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

Methods of data processing and statistical analysis

After compilation, the data was presented in the form of tables, figures and graphs, as necessary. Statistical analyses of the results were done by using computer based statistical software SPSS version 23. Means, standard deviations, percentage frequencies was determined as indicated.

RESULTS

From December 2019 to November 2020, a total 31 patients were included in the present study. There were 19 males and 12 females. The median age was 38 years, range 25-80 years. All patients had abdominal pain (100%), 23 (74.2%) had fever and 18 (58.1%) had jaundice. Comorbidities were present in 8 patients (DM: 5, HTN 3). ERCP stenting was done 5 (16.1%) patients (Table 1).

Table 1: Clinico-demographic characteristics of the patients

Variables	Values
Number of patients	31
Age in years, median (range)	38 (25-80)
Sex (M:F)	19:12
Clinical features	
Abdominal pain, n (%)	31(100%)
Fever, n (%)	23 (74.2%)
Jaundice, n (%)	18 (58.1%)
Comorbidities	
DM, n (%)	5 (16.1%)
HTN, n (%)	3 (9.67%)
ERCP	
Not done, n (%)	26 (83.9%)
Done with stenting, n (%)	5 (16.1%)

Table 2: Laboratory findings of 31 patients

Variables	Mean± SD	Range
WBC (/mm3)	12140±4002	5000-19000
S. Bilirubin (mg/dl)	3.4±4.2	0.30- 15.00
Alkaline phosphatase (U/L)	308.1± 305.2	70.0-1067.0
PT (sec)	12.7± 1.8	10.0-18.0
INR	1.03±0.12	0.83-1.3
S. Albumin (gm/dl)	3.5±.074	2.10- 4.80
CA 19.9 (U/L)	672.9±2770	2.0-15404.0

The mean WBC levels were 12140±4002/mm3 (range: 5000-19000/mm3). The liver function of all 31 patients were; serum bilirubin 3.4±4.2 mg/dl (range

0.30- 15.00 mg/dl), Alkaline phosphatase 308.1± 305.2 IU/L (range: 70- 1067IU/L), Prothrombin time 12.7± 1.8sec (range: 10-18 sec.), INR 1.03±0.12 (range 0.83-1.3) and serum albumin 13.2±16.1 gm/dl (2.1-48.0 gm/dl). The tumor CA 19.9 levels were 672.9±2770 U/L (range 2.0-15404 U/L) (Table 2).

Figure 1: Hepatolithiasis left lobe

Figure 2: Hepatolithiasis Both lobe

Figure 3: Left lateral Segment containing multiple stones

Table 3: Isolated organisms from bile (n=31)

Variables	N (%)
Total patients	31 (100%)
Culture positive	24 (77.4%)
Culture negative	7 (22.6%)
Isolated Organisms (n=24)	
E. coli	13/24 (54.17%)
Klebsiella spp.	6/24 (25.00 %)
Pseudomonas spp.	2/ 24 (8.33 %)
Acinetobacter spp.	1/24 (4.17%)
Staphylococcus aureus	1/24 (4.17%)
Salmonella typhi	1/ 24 (4.17%)

Organisms were grown in the bile of 24 patients (24/31, 77.4%), and organism were not grown in rest 7 patient's bile (22.6%). Among the isolated organisms, E. coli were found in 13 (54.17%), Klebsiella in 6 (25%), Pseudomonas in 2 (8.33%), Acinetobacter in 1 (4.17%), staphylococcus in 1 (4.17%) and Salmonella in 1 (4.17%) (Table 3).

Table 4: Antibiotic sensitivity pattern of bile culture

Antibiotic Group	Antibiotic	Sensitivity, N (%)	Resistance, N (%)
Penicillin	Amoxicillin	7/21 (33.3%)	14/21 (66.67%)
	Ticarcillin	2/2 (100%)	0/0 (0%)
	Tazobactam+ Piperacillin	16/22 (72.7%)	6/22(27.3%)
Cephalosporins	Cefuroxime	5/21 (23.8%)	16/21 (76.2%)
	Cefotaxime	7/22(31.8%)	15/22 (69.2%)
	Ceftriaxone	8/22(36.4%)	14/22 (63.6%)
	Ceftazidime	7/16(43.8%)	9/16(56.2%)
	Cefepime	6/9(66.67%)	3/9 (33.33%)
Aminoglycosides	Gentamicin	12/23(52.2%)	11/23/(47.8%)
	Netilmicin	2/3(66.67%)	1/3 (33.33%)
	Amikacin	19/23(82.6%)	4/23 (17.4%)
Carbapenem	Imipenem	7/7(100%)	0/0(0%)
	Meropenem	16/22(72.7%)	6/22 (27.3%)
	Aztreonam	3/11(27.3%)	8/11 (72.7%)
Fluoroquinolones	Ciprofloxacin	6/23(26.1%)	17/23 (75.9%)
	Ofloxacin	2/2 (100%)	0/0(0%)
	Levofloxacin	3/6 (50%)	3/6(50%)
Macrolides	Azithromycin	7/21(33.3%)	14/21 (66.67%)
Others	Cotrimoxazole	9/21(42.9%)	12/21 (57.1%)
	Doxycycline	5/6 (83.3%)	1/6(16.7%)
	Tigecycline	5/5 (100%)	0/0(0%)
	Colistin	23/23(100%)	0/0(0%)

In penicillin group, Tazobactam+Piperacillin were tested in 22 patients, this combination was sensitive to 16 (72.7%) and resistant to 6 (27.3%) patients. Ticarcillin was tested in 2 patients; both of them were sensitive to ticarcillin. In contrast amoxicillin was tested in 21 patients; it was sensitive to 7 (33.3%) patients only and resistant to 14 (66.7%) patients.

In Cephalosporins group – Cefepime was tested in 9 patients and sensitivity was 66.67%, ceftazidime (43.8%), Ceftriaxone (36.4%) sensitive. In Aminoglycosides group Amikacin was tested 23 patients and sensitivity was 82.6%. Gentamycin was tested similar number with sensitivity was 52.2%. In Carbapenem group imipenem was tested 7 out of 7, so sensitivity was 100%. Meropenem was tested in 22 patients and its sensitivity was 72.7%. In Fluoroquinolones group – Ciprofloxacin was examined 23 patients with 26.1% sensitivity.

In Macrolides group azithromycin was tested 21patients and sensitivity was 33.3%.

Doxycycline was examined 6 patients with 83.3% sensitivity and Tigecycline was tested 5 patients with 100% sensitivity. Colistin was found 100% sensitive in patients of 23 (Table 4).

DISCUSSION

In normal condition, human bile is sterile. But bile of hepatolithiasis is highly contaminated due to it's prolong stasis [8]. In our study, Positive bile culture

was found in 77.4% samples. This proportion may vary; from 67% [3] to 96.3% [10] to other study, but most frequent organisms found in this study was E. coli like our study. The bacteria in bile duct produce β-glucuronidase which can hydrolyze conjugated bilirubin to unconjugated bilirubin. Then, unconjugated bilirubin and ionized calcium will combine together and form calcium bilirubinate stone [1]. Other organisms isolated from bile have β-glucuronidase activity includes Klebsiella, Pseudomonas and other Enterobacteriaceae. In our study, E.coli (54.17%), Klebsiella spp (25.00%), Pseudomonas (8.33%), Acinetobacter (4.17%), Staphylococcus (4.17%), Salmonella (4.17%) which was similar to other study [9]. In Italy, a study revealed that the most commonly isolated bacteria were Enterococcus species, followed by Pseudomonas species, Escherichia coli species, Klebsiella species in decreasing order [4].

Antibiotic susceptibility pattern of isolates from bile in our study showed good sensitivity towards Colistin, imipenem and Tigecycline (100%), Doxycycline (83.3%), amikacin (82.6%), Meropenem & Tazobactam+ Piperacillin (72.7%), cefepime (66.7%). This findings was consistent with SMART study [5]. Antibiotic sensitivity pattern from bile isolates showed good sensitivity to meropenem (86%), imipenem (79%), amikacin (86%) and piperacillin / Tazobactam (61%) from a Turkey study [6] where most common organism was E. coli like our study. When individuals organism was assessed in term of sensitivity in our study, we found that E. coli isolates (n=13) showed good susceptibilities towards ceftazidime (57.1%), cefepime (50%), ticarcillin (100%), amikacin (92.3%), meropenem (83.3%), Tazobactam + piperacillin (84.6%), colistin (100%), imipenem (100%), doxycycline (83.3%), tigecycline (100%) and clindamycin (100%). A Philippian study reported sensitivity of E. coli isolated from bile where they found organisms were sensitive to ceftazidime (85%), cefepime (93%), amikacin (90%), meropenem (100%), Tazobactam + piperacillin (96%), imipenem (100%), very close to our study [7]. From this study we found that usual used antibiotic in our setting like cephalosporin and fluoquinolones are resistant to biliary organism in hepatolithiasis patients.

CONCLUSION

Cephalosporins and Fluoroquinolones are not good antibiotics for prophylactic measure in patients with hepatolithiasis in our setting. Imipenem, meropenem, doxycycline, amikacin, cefepime and Tazobactam/piperacillin can be used empirically in hepatolithiasis patients for the treatment of cholangitis.

REFERENCES

1. Bharti, J. N., Rani, P., Kamal, V., & AgARwAL, P. N. (2015). Angiogenesis in breast cancer and its correlation with estrogen, progesterone receptors and other prognostic factors. *Journal of Clinical and Diagnostic Research: JCDR*, *9*(1), EC05.
2. Catena, M., Aldrighetti, L., Finazzi, R., Arzu, G., Arru, M., Pulitanò, C., & Ferla, G. (2006). Treatment of non- endemic hepatolithiasis in a Western country. The role of hepatic resection. *The Annals of the Royal College of Surgeons of England*, *88*(4), 383-389. doi: 10.1308/003588406X98711.
3. Chang, W. T., Lee, K. T., Wang, S. R., Chuang, S. C., Kuo, K. K., Chen, J. S., & Sheen, P. C. (2002). Bacteriology and antimicrobial susceptibility in biliary tract disease: an audit of 10-years-experience. *The Kaohsiung Journal of Medical Sciences*, *18*(5), 221-228.
4. Clemente, G., De Rose, A. M., Murri, R., Ardito, F., Nuzzo, G., & Giuliante, F. (2016). Liver resection for primary intrahepatic stones: focus on postoperative infectious complications. *World Journal of Surgery*, *40*, 433-439.
5. Kim, H. J., Kim, J. S., Joo, M. K., Lee, B. J., Kim, J. H., Yeon, J. E., ... & Bak, Y. T. (2015). Hepatolithiasis and intrahepatic cholangiocarcinoma: a review. *World journal of gastroenterology*, *21*(48), 13418-13431. doi: 10.3748/wjg.v21.i48.13418.
6. Li, H., Zheng, J., Cai, J. Y., Li, S. H., Zhang, J. B., Wang, X. M., ... & Wang, G. S. (2017). Laparoscopic VS open hepatectomy for hepatolithiasis: An updated systematic review and meta-analysis. *World journal of gastroenterology*, *23*(43), 7791-7806. doi: 10.3748/wjg.v23.i43.7791.
7. Sakpal, S. V., Babel, N., & Chamberlain, R. S. (2009). Surgical management of hepatolithiasis. *Hpb*, *11*(3), 194-202. doi: 10.1111/j.1477-2574.2009.00046.x.
8. Sheen-Chen, S. M., Chen, W. J., Eng, H. L., Sheen, C. W., Chou, F. F., Cheng, Y. F., & Lee, T. Y. (2000). Bacteriology and antimicrobial choice in hepatolithiasis. *American journal of infection control*, *28*(4), 298-301.
9. Sheen-Chen, S. M., Chen, W. J., Eng, H. L., Sheen, C. W., Chou, F. F., Cheng, Y. F., & Lee, T. Y. (2000). Bacteriology and antimicrobial choice in hepatolithiasis. *American journal of infection control*, *28*(4), 298-301. doi: 10.1067/mic.2000.107071.
10. Tabata, M., & Nakayama, F. (1984). Bacteriology of hepatolithiasis. *Progress in Clinical and Biological Research*, *152*, 163-174.
11. Uchiyama, K., Ueno, M., Ozawa, S., Kiriyama, S., Kawai, M., Hirono, S., ... & Yamaue, H. (2011). Risk factors for postoperative infectious complications after hepatectomy. *Journal of Hepato-Biliary-Pancreatic Sciences*, *18*(1), 67-73. doi: 10.1007/s00534-010-0313-1.