



Cohort Study

Biliary infection; distribution of species and antibiogram study

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ABSTRACT

Background: Biliary infections like cholecystitis and cholangitis are common and could be life threatening if treated inappropriately. Prescribing antibiotics is the key to control such infections. Occurrence of bacterial resistance to antibiotics is highly probable and should be continuously monitored. This study aimed to re-evaluate bacterial species distribution and their interaction to antibiotics in biliary infections.

Method: Total 2288 patients who were diagnosed as whether acute or chronic cholecystitis with/without concurrent cholangitis enrolled in this cohort study. All were candidate for cholecystectomy operation. In the theatre a sterile bile sample was aspirated from the gallbladder as early as the organ was exposed. Analysis was performed on culture and antibiogram results.

Results: Finally 492 (21.5%) microorganism growth was seen in all culture environments. Bacterial colonization was most common in cholangitis (63.8%) which followed by acute (26%) and chronic (10.9%) cholecystitis respectively ($p = 0.001$). *Escherichia coli* (58%) and Klebsiella species (12.2%) were mostly isolated pathogens. Antibiogram study illustrated bacterial sensitivity of gram-negative pathogens to imipenem (100%), amikacin (98.1%), and gentamicin (90.4%) which in gram-positive bacterial species was 100% to imipenem, vancomycin, rifampicin, and clindamycin.

Conclusion: Cephalosporins as an empirical treatment for biliary infections is not suitable. Aminoglycosides including amikacin and gentamicin are costly beneficial as the first line for empirical antibiotic therapy in selected patients because of their good bacterial sensitivity and low expenses. Imipenem should remain for multidrug resistance species.

1. Introduction

Physiologic bile production, secretion, storage, exertion, and drainage occur through a sterile biliary system originates from hepatocytes and ends to ampulla of Vater located in second portion of duodenum. The mainstay of bile to remain sterile directly depends on continuous bile flow in biliary tree which includes intrahepatic, common hepatic (CHD), and common bile duct (CBD) in addition to gall bladder (GB) as a bile reservoir pouch. Gall stone formation is the most common cause for extra hepatic biliary obstruction which could follow by super imposed infective complication. The result of the latter could manifest as whether acute or chronic cholecystitis and life-threatening cholangitis [1–4]. Microbial studies on culture of bile sample which obtained from patients underwent cholecystectomy pointed to *Escherichia coli* (E.coli) as the most common colonized microorganism. Since biliary infection particularly in case of cholangitis could be settled to catastrophic outcomes like multi-organ failure which could be followed

by death, therefore currently prompt empirical antibiotic therapy in suspected patient with according biliary diagnosis is mandatorily indicated [5–7]. Empirical antibiotic regimen commonly includes of third generation cephalosporin or quinolones in combination with metronidazole which is currently recommended as the first line broad-spectrum medications for biliary infections. In similar to every other antibacterial agent drug resistant species generation is highly presumptive and should be meticulously monitored to avoid generation of drug resistant species. Considering the latter permanent control studies with goal of monitoring antibiotic resistance fundamentally through antibiogram tests is highly recommended. Paying insufficient attention to antibiotic resistance would impose large amount of expense and disaster to both the human being and health care systems all over the world. Considering aforementioned data, this study aimed to reevaluate bile culture characteristics in patients underwent cholecystectomy to assess if current recommended empirical antimicrobial regimen is impacting and costly beneficial.

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2. Materials and method

After obtaining ethical committee approval (IR.KAUMS.MEDNT.REC.1388.18) this cohort study was conducted with registration code IRT201203131102C31 which is available at www.irct.ir under direct performance and supervision of departments of general surgery, radiology, microbiology, and pathology of medical university from 2008 to 2020. Eventually 2288 of all 8721 patients were enrolled. For all participants history taking, clinical examination, and ultrasound study of biliary tree was accomplished by attributed physician. Acute cholecystitis (AC) was defined as patient's complaint of sustain abdominal right upper quadrant (RUQ) or epigastric pain for 24 h or more in addition to any positive clinical or radiologic evidence of Morphy's sign, gall stone (s) or sludge in GB, wall thickening (≥ 4 mm), layering or adjacent free fluid around GB in imaging study. Chronic cholecystitis (CC) diagnosis was reserved for repetitive colicky painful attacks or discomfort in RUQ or epigastrium after serving fatty meals without clinical or radiologic attributed findings mentioned for AC except for gall stone(s) or sludge. Cholangitis was defined as sustain RUQ or epigastric pain in addition to presence of Charcot's triad including of fever, local RUQ tenderness, and direct hyperbilirubinemia (conjugated bilirubin $\geq 15\%$ of total bilirubin) with evidence of gall stone in whether GB or CBD in imaging (US or Magnetic resonance cholangiopancreatography-MRCP) and raised serum alkaline phosphatase at least three times more than its normal level. Exclusion and inclusion criteria of the study are introduced in [Table 1](#).

All patients signed written consent form to participate. They underwent index cholecystectomy whether through open or laparoscopic surgical approach. In the theatre, 2 cc of bile fluid was extracted from GB through the fine needle aspiration (FNA) technique and sent to microbiology laboratory for culture sample in media. Staining, culture, and antibiogram tests were implemented for every bile sample. Applied culture environment was included blood, chocolate, and MacConkey agar. To separate gram positive (G+) pathogens Mannitol salt agar was enriched by adding catalase, coagulase, hippurate hydrolase, 6.5% salt, and bacitracin or optochin containing material. Identification of gram negative (G-) bacterial species was made on biochemical media involved sulfide indole motility, citrate, urea, lysine decarboxylase, and methyl

Table 1
Exclusion and inclusion criteria of the study.

Criterion	
Exclusive	Inclusive
Positive history of previous	1. Patients with diagnosis of AC, CC and cholangitis by
• Cholecystectomy	aforementioned characteristics
• ERCP ^a and CBD stent placement in ≥ 6 weeks from index operation	underwent index
• Sphincterotomy of Vater's ampulla in ≥ 6 weeks from index operation	cholecystectomy
• Gastrectomy; partial or total	2. Age ≥ 18
• Gastrostomy or jejunostomy feeding tube placement	3. Consent for participation
• Choledochoduodenostomy or Choledochojejunostomy	
Immunodeficiency	
• Uncontrolled diabetes mellitus; Hb A1c $\geq 8\%$	
• Active chemotherapy	
• HIV positive	
Complicated disease with	
• Gangrenous or empyematous GB; detected intraoperatively	
• Severe cholangitis with positive Reynold's pentad; hypotension and altered mental status added to Charcot's triad	
Suspicion of bile contamination during sampling procedure	

^a Endoscopic retrograde cholangiopancreatography.

red Voges Proskauer (MR-VP). Antibiogram test was accomplished through antibiotic disc diffusion approach (HiMedia®-India) on Mueller-Hinton or Trypticase agar. Antibiotic disc for G-microorganism was included of imipenem, piperacillin, ceftazidime, ceftriaxone, ceftizoxime, cefixime, cefepime, ciprofloxacin, gentamycin, and amikacin. For G+ germs antibiotic discs were penicillin, amoxicillin clavulanate, vancomycin, clindamycin, rifampin, imipenem, cefepime, tetracycline, erythromycin, ciprofloxacin, and cefotaxime. In order to assess sensitivity and resistance of microorganisms according to antibiogram study Clinical & Laboratory Standards Institute (CLSI) criteria was considered. Bacterial sensitivity was identified when no growth occurred in periphery of the antibiotic disc. Resistant pathogens didn't respect to presence of the antibiotic disc. Intermediate group was not completely sensitive or resistant to antibiotic and therefore generated smaller uninfected halo in peripheral zone of antibiotic disc than did sensitive bacterial species.

Data was analyzed using SPSS 20. Parametric values were described by numbers, mean, and standard deviation. To compare means the independent *t*-test and the ANOVA were used. Non-parametric values were introduced by frequency and percent. Analysis was performed via chi-square and *t*-test while significant *p* was assumed as less than 0.05. This study was prepared in lined with STROCSS criteria [8].

3. Results

Of all 2288 cholecystectomy operations 668 (29.2%) and 1620 (70.8%) were performed in male and female respectively ($p < 0.001$). patients were from 21 to 95 years with mean age of 48.3 ± 17.6 years ($p = 0.4$). Respectively 1762 (77%) and 526 (23%) laparoscopic and open cholecystectomy was accomplished ($p < 0.001$). According to preoperative diagnosis total 977 (42.7%) and 1176 (51.4%) patients suffered from AC and CC respectively while 135 (5.9%) persons were affected by cholangitis ($p < 0.01$). Demographic data of the study participants based on culture result was shown in [Table 2](#).

All patients were discharged after early recovery and there was no remarkable complication for report in follow up outpatient visit. Bile culture study was negative for growth of microorganism in 1796 (78.5%) media ($p < 0.01$). Results of bile culture study including of 492 (21.5%) positive growth was illustrated in detail by [Table 3](#).

Analysis showed that bacterial growth was highly suspected in cholangitis (63.8%) when compared with whether AC (26%), CC (10.9%) or even both (36.9%)($p = 0.001$). According to data in [Table 3](#) gram negative enterobacteriaceae(86.8%) was the mostly colonized species followed by gram positive(8.5%) and yeast(4.7%) ($p < 0.001$).

Analysis showed no significant difference between patients' gender and positive colonization ($p = 0.1$). Mean age of patients with negative culture was 47.4 ± 17 years while it was 51.5 ± 19.8 years for subjects with positive media ($p = 0.4$). All of colonies were filled with dominant one species of microorganism and no polymicrobial growth was detected ($p < 0.001$).

Antibiogram study revealed sensitivity of gram negative

Table 2
Demographic findings of all study participants based on culture result.

	n = 2288	Culture		P
		Positive(n = 492)	Negative(n = 1796)	
Gender	Male	117(23.8%)	551(30.7%)	0.1
BMI	Kg/m ²	$32.3 \pm 7.5^{\dagger}$	31.4 ± 6.6	0.07
Age	Year	51.5 ± 19.8	47.4 ± 17	0.4
Operation approach	Laparoscopic	324(65.8%)	1438(80%)	<0.001
Initial diagnosis	AC ^a	262(53.2)	715(39.8%)	<0.001
	CC**	144(29.3%)	1032(57.5%)	
	Cholangitis	86(17.5%)	49(2.7%)	

^a Acute cholecystitis **chronic cholecystitis \dagger mean \pm SD.

Table 3
Results of germ colonization in patients with biliary infection.

Germ	Culture								
	Positive						Negative	Total	
	Gram negative				Gram positive				
	Enterobacteriaceae				S.aureus		Yeast		
	E.coli	Klebseilla	Citrobacter	Others	S.aureus	Diphtheroid			
AC ^a	158(16.2)*	23(2.4)	9(0.9)	34(3.5)	14(1.4)	16(1.6)	8(0.8)	715(73.2)	977(42.7)
CC ^b	80(6.8)	17(1.4)	20(1.7)	0	12(1)	0	15(1.3)	1032(87.8)	1176(51.4)
Cholangitis	48(35.5)	24(18)	8(5.9)	6(4.4)	0	0	0	49(36.2)	135(5.9)
Total	286(12.5)	64(2.8)	37(1.6)	40(1.8)	26(1.1)	16(0.7)	23(1)	1796(78.5)	2288(100)

^a Acute cholecystitis.

^b Chronic cholecystitis * n(%).

enterobacteriaceae to imipenem, amikacin, and gentamycin was 100, 93.7, and 90.4% respectively. Considering gram positive germs sensitivity to antibiotics imipenem, vancomycin, rifampin, and clindamycin were all absolutely effective (100%) on prevention of colonization of these species. Tables 4 and 5 demonstrate findings related to antibiotic study considering bacterial reactions to antibiotics.

4. Discussion

Biliary infection is commonly expected to be found in according disease such as whether acute or chronic cholecystitis and cholangitis. Because of susceptibility of progression of biliary infection to overwhelming clinical condition particularly in case of cholangitis, therefore medical evidence indicates initiation of antibiotic medication when attributed diagnosis is suspected. Since generation of species with resistance to antibiotic is always possible therefor paying meticulous attention to this issue is highly important to prevent generation of such developed germ. Interestingly, this study has demonstrated that not only biliary infection is still prevalent and over 21% of attributed mild to moderate disease shows positive microorganism colonization in media but also bacterial reaction to cephalosporins which is currently recommended as the first line antibiotic regimen against such infection is changed and prevention of bacterial colonization with cephalosporins is not generally favorable.

Over 21% of all bile aspirated specimen was in association with microorganism colonization in this study. However, respectively about 36% and near 64% of patients with cholecystitis and cholangitis finally had positive culture. The amount of positive microorganism growth was reported by other authors more and from 40 to 50% in cholecystitis to 70% in cholangitis [5–7,9,10]. Commonly in almost all previous article and also in current one diagnosis of cholangitis was highly suggestive for bacterial colonization which lightened importance of proper antibiotic prescription. All of extracted colonies in this survey were monobacterial and no polymicrobial growth was detected. However similar studies claimed that polymicrobial colonization in a same sample was not uncommon and was remarkable in cholangitis [11–13]. The diversity for amount of positive cultures and also polymicrobial germ colonization could be related to difference in patient selection, study inclusion and exclusion criteria, and availability of hi-tech microbiology laboratory to prepare suitable in-vitro condition for anaerobe bacterial species to

growth in media. Our reference laboratory was not equipped with such hi-tech instruments. On the other hand severity of disease in current study was considered from mild to moderate and critical patient was not included.

In lined with previous reports this study also showed family of enterobacteriaceae specifically E.coli and klebsiella were the most common culprit pathogens for biliary infection [10–18]. In other studies the latter sequence was followed by pseudomonas aeruginosa The mostly accepted hypothesis for the latter is ascending immigration of adjacent intestinal normal flora through the biliary tree [3–7,12–22].

Antibiogram study was performed in extended burden of antibiotic discs for both gram positive and gram negative species in this report. This study found that G-bacterial species were highly sensitive to imipenem (100%), amikacin (93.7%), and gentamycin (90.4%) respectively. However, these species showed good resistance to cefepime (50%), ceftriaxone (54%), and cefixime (75%) respectively. Gram positive bacterial species demonstrated highly sensitive characteristic against imipenem, vancomycin, clindamycin, and rifampin (100%), although no favorable sensitivity was detected against other antibiotic discs like amoxicillin clavulanate (25%). Wang et al. claimed resistance of G-bacterial species increased against antibiotics subsequently from carbapenems (2.8–5.6%) and amikacin (28.7%) to penicillin, quinolones, and cephalosporins (<50%). Previous data showed G-bacterial species were not enough sensitive to clindamycin, ceftazidime, and ceftriaxone although quinolones and ampicillin inhibited the growth [3–7,9–21]. Authors also implied on that G+ bacterial species were incredibly sensitive to vancomycin and ticoplanin (100%) although over 40% of these species showed remarkable resistance to penicillin, quinolones, and clindamycin [13–16].

Such remarkable amount of sensitivity to carbapenems and aminoglycosides in addition to unfavorable resistance of common colonized germs in biliary infection to cephalosporins (advised as first line empirical antibiotic treatment for biliary infection) was established in this study similar to other advocates [18–21].

According to our knowledge from searching literature and in lined with current study results, imipenem was the mostly efficient antibiotic formula against almost all germs colonized in biliary tree [12–24].

Since this study and some other similar reports showed, although distribution of biliary germs has not obviously changed important signs of resistance to currently advised antibiotics was flashed. Therefore,

Table 4
Antibiogram findings for gram negative bacterial species.

n = 427	Antibiotic disc									
Antibiogram study	imipenem	piperacillin	ceftazidime	ceftriaxone	ceftizoxime	Cefixime	cefepime	Ciprofloxacin	gentamycin	amikacin
Sensitive	427(100)	115(26.9)	159(37.2)	49(11.5)	107(25)	25(5.8)	76(17.8)	197(46.2)	386(90.4)	400(93.7)
Intermediate	0	116(27.1)	87(20.4)	148(34.6)	123(28.8)	82(19.2)	137(32.1)	68(15.9)	19(4.5)	19(4.4)
Resistant	0	196(46)	181(42.4)	230(53.9)	197(46.2)	320(75)	214(50.1)	162(37.9)	22(5.1)	8(1.9)
P	<0.001	0.4	0.5	0.7	0.6	0.8	0.7	0.5	<0.001	<0.001

*n(%).

Table 5
Antibiogram findings for gram positive bacterial species.

n = 42	Antibiotic disc										
Antibiogram study	penicillin	Amoxicillin clavulanate	Vancomycin	clindamycin	Rifampin	imipenem	cefepime	tetracycline	Erythromycin	ciprofloxacin	cefotaxime
Sensitive	9(21.4) ^a	14(33.3)	42(100)	42(100)	42(100)	42(100)	11(26.2)	16(38.1)	6(14.3)	13(30.9)	15(35.7)
Intermediate	21(50)	10(23.8)	0	0	0	0	17(40.5)	4(9.5)	14(33.3)	7(16.7)	7(16.7)
Resistant	12(28.6)	18(42.9)	0	0	0	0	14(33.3)	22(52.4)	22(52.4)	22(52.4)	20(47.6)
P	0.6	0.5	<0.001	<0.001	<0.001	<0.001	0.4	0.4	0.4	0.4	0.5

^a n (%).

considering above results it is highly recommended to perform permanent multi center similar studies to carefully monitor changes of bacterial development specifically in case of resistance to antibiotics and revise attributed guidelines.

5. Limitation

This study was conducted in a single medical center. Specific regional nutritional behaviors could influence distribution of alimentary micro flora which was not considered in this study. Patient with severe or critical clinical condition was not included to participate. Lack of available hi-tech laboratory instruments led to miss in-vitro colonization and evaluation of anaerobe species.

6. Conclusion

Current for first line empirical antibiotic therapy –cephalosporin- in mild to moderate biliary infection seems to be faced with drug resistance and is not enough reliable. Aminoglycosides including amikacin and gentamycin are costly beneficial for empirical antibiotic therapy in selected patients with biliary infection. It is because of good bacterial coverage in addition to their low expense. Imipenem as the mostly potent antibiotic formula for growth inhibition of almost all biliary pathogens should remain for multidrug resistance species. It is advised to perform systematic reviews to revise current guideline for antibiotic prescription I biliary infection if needed.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Ethics approval and consent to participate

This study was performed under supervision of University of Medical Sciences and ethics committee has approved study design by registering code IR.KAUMS.MEDNT.REC.1388.18.

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Authors' contributions

SS: study design, supervision, interpret results.

SHR: study design, data collection.

AH: study design, data collection, data Analysis, interpret results, drafting article.

Consent for publication

Not applicable.

Availability of data and material

The data used to support findings of this study is not available in medical file archive.

Declaration of competing interest

All authors promised they have no private benefits for this study and all aspects of advantages referred back to Kashan University of medical sciences.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.amsu.2021.102822>.

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