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胆总管结石患者胆汁病原菌分布特点及耐药性分析*

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摘要 目的:探讨胆总管结石患者胆汁病原菌的分布特点以及耐药性的分析。**方法:**选择2016年6月-2017年6月期间我院收治的胆总管结石合并胆道感染患者160例为研究对象,所有患者均进行逆行内镜胆管造影(ERCP)并抽取胆汁标本,进行细菌培养和耐药性实验,评价分析胆汁病原菌的分布特点及耐药性情况。**结果:**160例患者中有117例(73.13%)检出病原菌,共培养出病原菌130株,其中有13例患者为两种病原菌同时感染。革兰阴性菌有95株(73.08%)、革兰阳性菌有31株(23.85%)、真菌有4株(3.08%)。比例由高到低的前六位病原菌依次为:大肠埃希菌、肺炎克雷伯菌、阴沟肠杆菌、屎肠球菌、铜绿假单胞菌、粪肠球菌。革兰阴性菌对亚胺培南、阿米卡星、美罗培南、他唑巴坦、头孢吡肟等三四代头孢菌素耐药率较低,对头孢曲松、环丙沙星、左氧氟沙星、哌拉西林、氨苄西林等耐药率较高。革兰阳性菌对替拉考宁、万古霉素、利奈唑胺等耐药率较低,对四环素、环丙沙星、左氧氟沙星、克林霉素、氨苄西林等耐药率较高。真菌对酮康唑、伊曲康唑、氟康唑等耐药率较低,对两性霉素B耐药率较高。**结论:**胆总管结石患者胆汁病原菌主要为革兰阴性菌、其次为革兰阳性菌,各病原菌对各种抗菌药物表现出不同的耐药性,因此在临床治疗时应参考药敏试验结果进行合理选择治疗药物。

关键词:胆总管结石;胆道感染;病原菌分布;抗菌药;耐药性

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Distribution of Bile Pathogens and Analysis of Drug Resistance in Patients with Choledocholithiasis*

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ABSTRACT Objective: To investigate the distribution characteristics of bile pathogenic and analysis of drug resistance in patients with choledocholithiasis. **Methods:** A total of 160 cases of choledocholithiasis combined with biliary tract infection, who were treated in the First Affiliated Hospital of Chengdu Medical College from June 2016 to June 2017, were selected as research subjects. All the patients were treated with retrograde endoscopic cholangiopancreatography (ERCP) and extracted the bile samples and bacterial culture and drug resistance experiment were conducted, the distribution characteristics and drug resistance of bile pathogens were analyzed. **Results:** Among 160 patients, 117 patients (73.13%) were detected pathogenic bacteria, and 130 bacteria were cultured, of which 13 were infected by two bacteria; there were 95 strains of Gram-negative bacteria (73.08%), 31 strains of Gram-positive bacteria (23.85%), 4 strains of fungi (3.08%). The first six pathogens from high to low were in turn: *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. The resistance rates of Gram-negative bacteria to imipenem, meropenem, Amikacin, tazobactam, cefepime and three or four generation cephalosporin were lower, the resistance rates to ceftazidime, ciprofloxacin, levofloxacin, piperacillin and ampicillin were higher. The resistance rates of Gram positive bacteria to Lincosamides, vancomycin, linezolid and other were lower, the resistance rates to tetracycline, ciprofloxacin, levofloxacin, clindamycin and ampicillin were higher. The resistance rates of fungi to ketoconazole, itraconazole and fluconazole were lower, and the resistance rate to amphotericin B was higher. **Conclusion:** The main pathogenic bacteria in the bile of patients with choledocholithiasis are Gram-negative bacteria, the second is Gram-positive bacteria. The pathogenic bacteria showed different resistance to various antibacterials; therefore, in the clinical treatment, drugs should be reasonably chosen according to the results of drug sensitivity test.

Keywords: Choledocholithiasis; Choledochic infection; Fistribution of pathogenic bacteria; Antimicrobial agents; Drug resistance

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前言

胆总管结石是指位于胆总管内的结石,大多数为胆色素结

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石或以胆色素为主的混合结石,好发于胆总管下端^[1-3]。其临床表现主要是胆总管梗阻和相伴发生的急性化脓性胆管炎,其中胆总管结石合并胆道感染是胆道系统最为常见的多发性疾病,随着细菌的大量繁殖,可能引起菌血症、脓毒血症等临床恶性事件,对患者生命健康造成严重威胁^[4-6]。临床上对于胆道感染患者多数均采用抗生素类药物进行抗感染治疗,在治疗过程中由于胆道中各病原菌分布情况的不同、主治医师对抗生素使用习惯的差异性及抗生素应用过程中的滥用现象,导致患者在治疗时易产生耐药菌株,影响了抗生素类药物的治疗效果,使得胆总管结石合并胆道感染的临床治疗成为一个棘手的问题^[7-9]。为此,如何选择合适的抗生素以取得更好的抗菌效果已成为临床关注的重点^[10,11]。本院近几年也陆续开展了胆总管结石合并胆道感染的抗菌治疗研究,通过对胆道感染病原菌的检查分类,以及及时了解胆总管结石患者胆汁标本的病原微生物学分布,并通过对各细菌的培养及耐药性实验,为临床胆总管结石患者的抗菌治疗提供科学合理的指导性建议,现作如下报道。

1 资料与方法

1.1 一般资料

以2016年6月-2017年6月期间我院收治的胆总管结石合并胆道感染患者160例为研究对象,纳入标准^[12]:(1)患者经多普勒超声诊断确诊为胆总管结石,并经血常规检查及细菌性培养试验确诊为胆道感染;(2)患者症状表现为发热、畏寒、黄疸或上腹部不同程度疼痛感;(3)患者身体状态良好,能够耐受逆行内镜胆管造影术(endoscopic retrograde cholangiopancreatography, ERCP)的检查;(4)患者对相关治疗药物无严重过敏反应及禁忌症;(5)患者签署知情同意书,研究方案经医院伦理学委员会批准。排除标准:(1)严重心、肝、肾功能不全者;(2)入院前已使用抗生素类药物进行治疗者;(3)精神状态异常者。其中男89例、女71例,年龄29-67岁,平均(48.92±10.21)岁。在患者进

行 ERCP 检查时采集患者的胆汁标本进行病原菌的检查分析。

1.2 研究方法

1.2.1 胆汁标本采集 在患者进行 ERCP 检查时采集患者的胆汁标本,具体操作方法为:应用 ED730 型十二指肠镜(日本富士株式会社)进行 ERCP 检查,导管消毒后经十二指肠乳头插入患者胆道内,在造影开始前采用无菌注射器抽吸患者胆汁约 5 mL,置于无菌试管内。所取胆汁标本在 2 h 内接种完毕,于 37℃ 恒温箱中进行孵育 24 h,然后进行细菌培养和耐药性试验。

1.2.2 病原菌分离鉴定及耐药性实验 病原菌的分离、鉴定按《全国临床检验操作规程》的相关要求进行操作,病原菌耐药性实验采用 Kirby-Bauer 纸片扩散法试验进行检测分析,用于定性的质控菌株为大肠埃希菌(ATCC25922)、金黄色葡萄球菌(ATCC25923)、铜绿假单胞菌(ATCC27853),判断标准参考美国临床实验室国家标准化委员会(National Standardization Committee of the clinical laboratory of the United States, NCCLS)公布的相关标准^[13],采用 VITEK 2 Compact 型全自动细菌鉴定及药敏分析系统(法国梅里埃公司)检测分析病原菌。

1.3 统计学方法

应用 SPSS 21.0 进行数据处理与分析,计数资料采用病例数或菌株数及所占百分比表示。

2 结果

2.1 胆汁病原菌分布特点

160 例患者中有 117 例(73.13%)检出病原菌,共培养出病原菌 130 株,其中有 13 例患者为两种病原菌同时感染。革兰阴性菌有 95 株(73.08%)、革兰阳性菌有 31 株(23.85%)、真菌有 4 株(3.08%)。比例由高到低的前六位病原菌依次为:大肠埃希菌、肺炎克雷伯菌、阴沟肠杆菌、屎肠球菌、铜绿假单胞菌、粪肠球菌,见表 1。

表 1 130 株病原菌分布特点及构成比例

Table 1 Distribution characteristics and proportion of 130 strains of pathogenic bacteria

Pathogenic bacteria	Bacterial strain	Proportion of strains(%)
Gram-negative bacteria	95	73.08
<i>Escherichia coli</i>	30	23.08
<i>Klebsiella pneumoniae</i>	21	16.15
<i>Enterobacter cloacae</i>	18	13.85
<i>Pseudomonas aeruginosa</i>	12	9.23
<i>Stenotrophomonas maltophilia</i>	7	5.38
<i>Klebsiella oxytoca</i>	4	3.08
<i>Acinetobacter Bauman</i>	3	2.31
Gram-positive bacteria	31	23.85
<i>Enterococcus faecium</i>	14	10.77
<i>Enterococcus faecalis</i>	10	7.69
<i>Staphylococcus aureus</i>	4	3.08
<i>Enterococcus bird</i>	2	1.54
<i>Streptococcus mitior</i>	1	0.77
Fungi	4	3.08
<i>Candida albicans</i>	2	1.54
<i>Candida vagitis</i>	2	1.54

2.2 主要革兰阴性菌对常规抗菌药物耐药性分析

主要革兰阴性菌对亚胺培南、阿米卡星、美罗培南、他唑巴

坦、头孢吡肟等三四代头孢菌素耐药率较低,对头孢曲松、环丙

沙星、左氧氟沙星、哌拉西林、氨苄西林等耐药率较高,见表 2。

表 2 主要革兰阴性菌对常规抗菌药物耐药性分析

Table 2 Analysis of the resistance of major gram-negative bacteria to conventional antibiotics

Antiseptic drugs	<i>Escherichia coli</i> (n=30)		<i>Klebsiella pneumoniae</i> (n=21)		<i>Enterobacter cloacae</i> (n=18)		<i>Pseudomonas aeruginosa</i> (n=12)	
	Drug-resistant strain	Drug resistance rate (%)	Drug-resistant strain	Drug resistance rate (%)	Drug-resistant strain	Drug resistance rate (%)	Drug-resistant strain	Drug resistance rate (%)
Imipenem	0	0.00	0	0.00	1	5.56	2	16.67
Amikacin	0	0.00	1	4.76	0	0.00	3	25.00
Meropenem	1	3.33	2	9.52	2	11.11	4	33.33
Tazobactam	2	6.67	4	19.05	3	16.67	5	41.67
Cefepime	3	10.00	5	23.81	6	33.33	4	33.33
Ceftazidime	4	13.33	7	33.33	5	27.78	6	50.00
Aztreonam	7	23.33	8	38.10	7	38.89	7	58.33
Gentamicin	12	40.00	9	42.86	6	33.33	8	66.67
Tobramycin	16	53.33	9	42.86	8	44.44	8	66.67
Cefuroxime	17	56.67	10	47.62	9	50.00	7	58.33
Ceftriaxone	19	63.33	12	57.14	11	61.11	7	58.33
Ciprofloxacin	20	66.67	7	33.33	12	66.67	4	33.33
Levofloxacin	21	70.00	8	38.10	9	50.00	4	33.33
Piperacillin	25	83.33	18	85.71	16	88.89	10	83.33
Ampicillin	29	86.67	20	95.24	14	77.78	12	100.00

2.3 主要革兰阳性菌对常规抗菌药物耐药性分析

主要革兰阳性菌对替拉考宁、万古霉素、利奈唑胺等耐药

率较低,对四环素、环丙沙星、左氧氟沙星、克林霉素、氨苄西林

等耐药率较高,见表 3。

表 3 主要革兰阳性菌对常规抗菌药物耐药性分析

Table 3 Analysis of the resistance of major gram-positive bacteria to conventional antimicrobial agents

Antiseptic drugs	<i>Enterococcus faecium</i> (n=14)		<i>Enterococcus faecalis</i> (n=10)	
	Drug-resistant strain	Drug resistance rate(%)	Drug-resistant strain	Drug resistance rate(%)
Teicoplanin	0	0.00	0	0.00
Vancomycin	1	7.14	1	10.00
Linezolid	1	7.14	1	10.00
Gentamicin	4	28.57	3	30.00
Ampicillin	5	35.71	4	40.00
Penicillin G	6	42.86	2	20.00
Tetracycline	7	50.00	4	40.00
Ciprofloxacin	9	64.29	5	50.00
Levofloxacin	10	71.43	6	60.00
Clindamycin	12	85.71	9	90.00
Ampicillin	13	92.86	8	80.00

2.4 真菌对常规抗菌药物耐药性分析

真菌对酮康唑、伊曲康唑、氟康唑等耐药率较低,对两性霉素 B 耐药率较高,见表 4。

些病理情况下诸如胆道结石、胆道梗阻、胆道内瘘、Oddi 括约肌等因素作用下易导致患者的体内的菌群发生异常现象,导致患者的肝脏、胆道内出现大量的致病菌,从而引发患者的胆道感染^[14-16]。经多数病原菌鉴定试验表明,胆道感染的致病菌种类与肠道菌群基本一致,基本表明胆道感染细菌是经肠道菌群逆行而引发的感染^[17-19]。目前,对于胆道感染的治疗以抗生素治疗

3 讨论

在正常健康人群中胆道内的胆汁通常是无菌的,但是在某

表 4 真菌对常规抗菌药物耐药性分析

Table 4 Analysis of the resistance of fungi to conventional antimicrobial agents

Antiseptic drugs	<i>Candida albicans</i> (n=2)		<i>Candida vagitis</i> (n=2)	
	Drug-resistant strain	Drug resistance rate(%)	Drug-resistant strain	Drug resistance rate(%)
Ketoconazole	0	0.00	0	0.00
Itraconazole	0	0.00	0	0.00
Fluconazol	0	0.00	0	0.00
Nysfungin	1	50.00	1	50.00
Amphotericin B	2	100.00	2	100.00

为主,但抗菌药的应用受到细菌耐药性的影响,使得治疗效果仍不能让广大医生和患者满意^[20-23]。分析其原因主要有以下两点,首先,不同患者胆总管结石胆汁内病原菌的菌群分布特点各异,而不同的抗生素具有不同的抗菌谱,这样有可能造成在不清楚患者具体胆汁菌群分布时的抗生素的盲目应用。其次,目前的抗生素种类繁多,医师在选择抗生素时往往按照自己的习惯方式用药,也容易导致对患者使用了不合适的抗菌药物,从而影响患者的治疗效果,并引起患者耐药菌株的出现^[24]。因而,对于此类患者在给予抗生素治疗前进行胆汁病原菌的菌群分布检测及耐药性试验是十分必要的,对临床用药选择有着重要的参考意义。

在本研究中对我院收治的胆总管结石合并胆道感染的患者进行病原菌分布检测和耐药性实验,根据检测结果显示我院胆总管结石患者胆汁中分布的病原菌分布特点为:菌群分布中以革兰阴性菌居多,占 73.08%,其次为革兰阳性菌,占 23.85%,也有个别病例为真菌感染,占 3.08%,表明革兰阴性菌已成为胆道感染的最主要的致病菌。本院患者中有 13 例患者为两种病原菌同时感染,即患者胆汁内同时检出革兰阴性菌和革兰阳性菌,分析其原因可能是由于患者长时间住院,病情经久不愈,胆道感染的反复发作而导致不同类型细菌的感染^[25,26]。革兰阴性菌中菌株比例较高的分别为大肠埃希菌、肺炎克雷伯菌、阴沟肠杆菌,革兰阳性菌中菌株比例较高的分别为屎肠球菌、粪肠球菌,结果显示我院患者胆汁病原菌中肠球菌所占比例较高,已成为胆道感染的主要的致病菌,可能与临床抗菌药的广泛应用以及滥用导致的细菌谱的变迁等因素有关^[27,28],近些年临床上大量应用的一代、二代头孢菌素类抗生素,使得多数的革兰阴性菌产生耐药性,不易被清除,在机体内的大量繁殖,造成其比例较高。耐药性检测方面,主要革兰阴性菌对亚胺培南、阿米卡星、美罗培南、他唑巴坦、头孢吡肟等三四代头孢菌素耐药率较低,对头孢曲松、环丙沙星、左氧氟沙星、哌拉西林、氨苄西林等耐药率较高,在革兰阴性菌里铜绿假单胞菌对各种抗菌药的耐药率均较高。主要革兰阳性菌对替拉考宁、万古霉素、利奈唑胺等耐药率较低,对四环素、环丙沙星、左氧氟沙星、克林霉素、氨苄西林等耐药率较高,说明革兰阴性菌和革兰阳性菌均对第一、二代头孢菌素及喹诺酮类抗菌药有很高的耐药性,这是因为第一、二代头孢菌素的抗菌谱较窄,在临床应用时间长、范围广,使得多数细菌对于此类药物均有一定的耐药性,同时,喹诺酮类抗菌药是广谱抗菌药,在临床上应用范围和频率极高,长期大量的使用喹诺酮类抗菌药导致患者对于此类抗菌药

出现严重的耐药性^[29]。此外,真菌对酮康唑、伊曲康唑、氟康唑等耐药率较低,对两性霉素 B 耐药率较高,对于真菌感染的胆总管结石患者,可采用酮康唑、伊曲康唑、氟康唑进行抗菌治疗。因此,在今后胆总管结石合并胆道感染的抗菌治疗过程中,医师应根据患者体内病原菌的不同,制定相应的抗菌药物治疗方案,患者切勿自行选择抗菌药物治疗,以避免抗生素的滥用。综合来看,本院胆总管结石患者胆汁病原菌分布特点及耐药性与临床报道结果基本相似^[30]。

综上所述,胆总管结石患者胆汁病原菌分布以革兰阴性菌为主,各病原菌对各种抗菌药物表现出现不同的耐药性,革兰阴性菌对亚胺培南、阿米卡星、美罗培南、他唑巴坦、头孢吡肟等三四代头孢菌素耐药率较低,革兰阳性菌对替拉考宁、万古霉素、利奈唑胺等耐药率较低,因此患者在治疗前应先确定感染的病原菌种类再制定相应的抗生素治疗方案。

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