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Analysis of Common Pathogenic Bacteria and Drug Resistance of Biliary Tract Infection in Nanjing Area

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Abstract **Background:** Analyze the distribution and drug resistance of pathogenic bacteria that cause biliary tract infections in Nanjing, and provide evidence for the rational use of antibacterial drugs in clinical practice. **Methods:** Clinical strains isolated from bile specimens of patients suspected of biliary infection in the First Affiliated Hospital of Nanjing Medical University in 2019 were collected, The drug susceptibility criteria are based on the standards published by the National Standardization Committee of the US Clinical Laboratories. WHONET 5.6 software was used to analyze the distribution of pathogens and drug resistance. **Results:** A total of 693 strains of pathogenic bacteria were isolated, including 448 Gram-negative bacteria(64.6%), 245 Gram-positive bacteria (35.4%). The top three pathogens were 210 strains of Escherichia coli(30.3%), 87 strains of enterococcus faecium (12.6%), 76 strains of klebsiella pneumoniae (11.0%), The resistance rates of Escherichia coli to ampicillin, cefuroxime, cefazolin, ceftriaxone, piperacillin and ampicillin / sulbactam were 80.1%, 69.4%, 67.3%, 64.1%, 63.6% and 62.8%, The resistance rates of Klebsiella pneumoniae to ampicillin / sulbactam, cefuroxime and cefazolin were 65.8%, 64.5% and 61.1%, The resistance rates of Enterobacter cloacae to ceftriaxone, ceftazidime and aztreonam were 56.2%, 53.1% and 53.1%, The resistance rates of Enterococcus faecium to moxifloxacin, clindamycin, erythromycin, penicillin G, ampicillin, ciprofloxacin and levofloxacin were 100%, 90%, 76%, 72.1%, 64.4%, 64% and 62%. **Conclusions:** Pathogens of biliary tract infections are mainly Enterobacteriaceae such as Escherichia coli and Klebsiella pneumoniae, followed by Enterococcus faecium and Enterococcus faecalis. There were many drug-resistant bacteria, so we should pay attention to bile specimen culture and drug sensitivity test.

Keywords: *Biliary tract infection, pathogenic bacteria, drug resistance, Nanjing*

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1. Introduction

Biliary tract infection is a common multiple refractory disease in surgical abdominal infection [1,2,3]. Severe infection can cause systemic inflammatory response syndrome, sepsis, multiple organ dysfunction, and even death. Identification of pathogenic microorganisms is the key to the treatment of biliary tract infections, and identification of pathogens and their drug resistance is an important basis for treatment [4,5,6]. The pathogenic bacteria and drug resistance of biliary tract infection were different in different regions and different periods. In the era of increasing antimicrobial resistance, monitoring and updating the local antimicrobial spectrum has become an urgent task.

Understanding of the local pathogenic bacteria causing biliary tract infection and drug resistance status, It is of great significance to choose antibiotics for clinical empirical treatment of biliary tract infection [7]. Therefore, this study analyzed pathogenic bacteria isolated from bile samples of patients with biliary tract infection and their drug resistance in the first Affiliated Hospital of Nanjing Medical University in 2019, so as to provide evidence for the treatment of biliary tract infection.

2. Materials and Methods

2.1. Source of Bacterial Strain

Clinical strains isolated from bile specimens of patients suspected of biliary infection in the First Affiliated Hospital of Nanjing Medical University in 2019 were collected, A total of 693 strains were collected. Excluding duplicate strains, a total of 693 strains of bacteria were collected. The biliary diseases include bile duct stones, gallstones, acute cholecystitis, chronic cholecystitis, acute cholangitis, chronic cholangitis, bile duct tumors, pancreatic cancer and gallbladder cancer. The quality control strains are Escherichia coli ATCC25922, Pseudomonas aeruginosa ATCC27853, Staphylococcus aureus ATCC29213, Enterococcus faecalis ATCC29212 preserved in the Microbiology Laboratory of the First Affiliated Hospital of Nanjing Medical University.

2.2. Instruments and Reagents

French Mérieux VITEK 2 COMPACT automatic microbial identification and drug sensitivity analysis system and its supporting reagents. Merieux VITEK MS automatic rapid microbial mass spectrometry detection system and its supporting reagents, Blood agar plate, McConkey agar plate, chocolate agar plate and fungus culture plate produced by Zhengzhou Antu company.

2.3. Bile Specimen Collection

Bile samples were collected by surgical methods, extracted aseptically, injected into aseptic bottles, and immediately sent to the microbiology room of the laboratory for inoculation and culture.

2.4. Pathogenic Bacteria Culture and Drug Sensitivity Identification Test

The bile samples were centrifuged and the sediment was inoculated on blood plate, McConkey plate and chocolate plate, Bacteria isolation and identification are carried out in strict accordance with the "National Clinical Laboratory Procedures", The isolated strains were identified by virek 2compact automatic bacterial identification system. The quality control strains were Escherichia coli ATCC25922, Staphylococcus aureus atcc29213, Enterococcus faecalis ATCC29212 and Pseudomonas aeruginosa ATCC27853. The drug susceptibility criteria are based on the standards published by the National Standardization Committee of the US Clinical Laboratories.

2.5. Statistical Processing

WHONET 5.6 software was used to analyze the distribution of pathogens and drug resistance.

3. Result

3.1. Composition of Pathogenic Bacteria

A total of 693 strains of pathogenic bacteria were isolated, including 448 Gram-negative bacteria (64.6%), 245 Gram-positive bacteria(35.4%). The top three pathogens were 210 strains of Escherichia coli (30.3%), 87 strains of enterococcus faecium (12.6%), 76 strains of klebsiella pneumoniae (11.0%), see in Table 1.

3.2. Drug Resistance Rate of Gram Negative Bacteria

The resistance rates of Escherichia coli to ampicillin, cefuroxime, cefazolin, ceftriaxone, piperacillin and ampicillin / sulbactam were 80.1%, 69.4%, 67.3%, 64.1%, 63.6% and 62.8%, The resistance rate of Escherichia coli to amikacin, cefotetan, imipenem, meropenem and piperacillin / tazobactam was low(<10%).

The resistance rates of Klebsiella pneumoniae to ampicillin / sulbactam, cefuroxime and cefazolin were 65.8%, 64.5% and 61.1%, The resistance rate of Klebsiella pneumoniae to amikacin was low (12.3%).

The resistance rates of Enterobacter cloacae to ceftriaxone, ceftazidime and aztreonam were 56.2%, 53.1% and 53.1%, respectively, The resistance rate of Enterobacter cloacae to cefepime, gentamicin, imipenem, meropenem, sulfamethoxazole and tobramycin was low (< 10%).

The resistance rates of Citrobacter to cefazolin, cefuroxime, cefotetan, ceftriaxone, ceftazidime and aztreonam were 100%, 80%, 60%, 60%, 60% respectively, The resistance rate of Citrobacter to imipenem, meropenem, tobramycin and piperacillin / tazobactam was low (< 10%). see in Table 2.

Table 1. Microbial growth of bile culture

Bile	strains (n)	Proportion (%)	Bile	Strains (n)	Proportion (%)
Gram negative bacilli			Gram positive cocci		
Enterobacteriaceae			Enterococcus		
Gram negative bacilli			Enterococcus faecium	87	12.6
Escherichia coli	210	30.3	Enterococcus faecalis	71	10.2
Klebsiella pneumoniae	76	11.0	Enterococcus gallinarum	11	1.6
Other Klebsiella species	19	2.7	Enterococcus casseliflavus	11	1.6
Enterobacter cloacae	32	4.6	Other enterococci	18	2.6
Citrobacter	22	3.2	Staphylococcus		
Proteus	6	0.9	Staphylococcus haemolyticus	9	1.3
Morganella morganii	3	0.4	Staphylococcus aureus	5	0.7
Pandora	3	0.4	Staphylococcus epidermidis	3	0.4
Escherichia hermannii	1	0.1	Other Staphylococcus	3	0.4
Salmonella London	1	0.1	Streptococcus		
Other genera of Enterobacteriaceae	13	1.9	Streptococcus salivarius	6	0.9
Nonfermenters			Bradycardia / oral Streptococcus	4	0.6
Acinetobacter baumannii	19	2.7	α- hemolytic streptococcus	4	0.6
Other Acinetobacter species	5	0.7	Streptococcus sanguis	4	0.6
Pseudomonas aeruginosa	20	2.9	Streptococcus pharyngitis	3	0.4
Other Pseudomonas	4	0.6	Streptococcus pneumoniae	1	0.1
Stenotrophomonas maltophilia	8	1.2	Other Streptococcus	5	0.7
Some species of Alcaligenes	2	0.3			
Shewanella putrefaciens	2	0.3			
Shewanella algae	2	0.3			

Table 2. Drug resistance rate of Gram-negative bacilli

Antibacterials	Escherichia coli n=210				Klebsiella pneumoniae n=76			
	n	Resistance	Medium	Sensitive	n	Resistance	Medium	Sensitive
Amikacin	204	2	0	98	73	12.3	0	87.7
Ampicillin	206	80.1	4.4	15.5	/	/	/	/
Aztreonam	210	54.33	0.47	45.19	76	46.1	0	53.9
Ceftazidime	209	37.8	1	61.2	76	41.3	1.5	57.2
Ciprofloxacin	206	54.4	2.4	43.2	76	38.2	0	61.8
Cefatriaxone	206	64.1	1.5	34.5	76	53.9	0	46.1
Cefoperazone / sulbactam	53	13.2	15.1	71.7	14	42.9	35.7	21.4
Cefotetan	206	6.8	2.4	90.8	76	22.4	1.3	76.3
Cefuroxime	209	69.4	7.2	23.4	76	64.5	5.3	30.3
Cefazolin	199	67.3	0	32.7	72	61.1	0	38.9
Cefepime	210	32.3	7.2	60.5	76	31.6	5.3	63.2
Gentamicin	210	21.8	1.5	76.7	76	32.9	2.6	64.5
Imipenem	210	4.3	0	95.7	76	20	0	80
Levofloxacin	210	49.5	3.8	46.7	76	32.9	1.3	65.8
Meropenem	210	3.3	0.5	96.2	76	20	0	80
Piperacillin	206	63.6	8.7	27.7	76	53.9	11.8	34.2
Ampicillin / sulbactam	210	62.8	11.9	25.3	76	65.8	2.6	31.6
Compound sulfamethoxazole	210	44.3	0	55.7	76	43.4	0	56.6
Tobramycin	206	10.2	13.6	76.2	76	25	11.8	63.2
Piperacillin / tazobactam	210	7.2	5.6	87.2	76	27.6	1.3	71.1
Antibacterials	Enterobacter cloacae n=32				Citrobacter n=22			
	n	Resistance	Medium	Sensitive	n	Resistance	Medium	Sensitive
Amikacin	31	0	0	100	18	0	0	100
Ampicillin	/	/	/	/	/	/	/	/
Aztreonam	32	53.1	0	46.9	20	60	0	40
Ceftazidime	32	53.1	0	46.9	20	60	0	40
Ciprofloxacin	32	18.8	0	81.2	20	15	10	75
Cefatriaxone	32	56.2	0	43.8	20	60	5	35
Cefoperazone / sulbactam	11	18.2	9.1	72.7	6	16.7	50	33.3
Cefotetan	/	/	/	/	5	60	0	40
Cefuroxime	14	35.7	35.7	28.6	5	80	0	20
Cefazolin	/	/	/	/	5	100	0	0
Cefepime	32	9.4	6.2	84.4	20	10	5	85
Gentamicin	32	6.2	0	93.8	20	10	0	90
Imipenem	32	6.3	6.2	87.5	18	5	5	90
Levofloxacin	32	15.6	3.1	81.2	20	15	0	85
Meropenem	32	0	0	100	20	5	0	95
Piperacillin	32	31.2	18.8	50	20	55	10	35
Ampicillin / sulbactam	/	/	/	/	/	/	/	/
Compound sulfamethoxazole	32	9.4	0	90.6	20	15	0	85
Tobramycin	32	0	9.4	90.6	20	5	10	85
Piperacillin / tazobactam	32	12.5	28.1	59.4	19	5.3	36.8	57.9

3.3. Drug Resistance Rate of Gram Positive Bacteria

The resistance rates of Enterococcus faecium to moxifloxacin, clindamycin, erythromycin, penicillin G, ampicillin, ciprofloxacin and levofloxacin were 100%, 90%, 76%, 72.1%, 64.4%, 64% and 62%, The resistance rate of Enterococcus faecium to linezolid, vancomycin and quinupristin / dafeptine was low (< 10%).

The drug resistance rates of Enterococcus faecalis to clindamycin and Quinuptin / daptin were 91.7% and 78.9%, The resistance rate of Enterococcus faecalis to penicillin G, ampicillin, linezolid and vancomycin was low (< 10%), see in Table 3.

3.4. Drug Resistance Rate of Non Fermentative Bacteria

The resistance rates of Acinetobacter baumannii to cephalosporins, carbapenems and Cefoperazone / sulbactam were over 60%, The resistance rate of Acinetobacter baumannii to amikacin was low. The resistance rate of Pseudomonas aeruginosa to ampicillin / sulbactam and cotrimoxazole was 100%, Pseudomonas aeruginosa had low resistance to amikacin and Cefoperazone / sulbactam.

Table 3. Drug resistance rate of Gram-positive cocci

Antibacterials	Enterococcus faecium n=87				Enterococcus faecalis n=71			
	n	Resistance	Medium	Sensitive	n	Resistance	Medium	Sensitive
Penicillin G	86	72.1	0	27.9	71	2.8	0	97.2
Ampicillin	87	64.4	0	35.6	71	1.4	0	98.6
High concentration gentamicin	87	34.5	0	65.5	70	17.1	0	82.9
High concentration streptomycin	86	31.4	/	0	70	24.3	0	75.7
Ciprofloxacin	86	64	/	8.1	71	15.5	1.4	83.1
Levofloxacin	87	62	10.4	27.6	71	14.1	1.4	84.5
Moxifloxacin	10	100	0	0	12	16.7	8.3	75
Clindamycin	10	90	0	10	12	91.7	0	8.3
Erythromycin	87	76	13.8	9.2	71	42.3	43.7	14.1
Linezolid	85	0	0	100	64	1.6	1.6	96.8
Vancomycin	87	0	1.1	98.9	70	0	0	100
Quinuptin / daptin	86	3.5	1.2	95.3	71	78.9	11.3	9.9
Tetracycline	86	24.4	0	75.6	71	52.1	0	47.9

Table 4. Drug resistance rate of Non fermentative bacteria

Antibacterials	Acinetobacter baumannii n=19				Pseudomonas aeruginosa n=20			
	n	Resistance	Medium	Sensitive	n	Resistance	Medium	Sensitive
Amikacin	3	0	0	100	20	0	5	95
Ceftazidime	19	68.4	10.5	21.1	20	35.01	5.04	60.05
Ciprofloxacin	19	63.2	0	36.8	20	9.99	0	90.01
Cefatriaxone	19	68.4	26.3	5.3		/	/	/
Cefoperazone / sulbactam	4	75	0	25	5	0	60	40
Cefepime	19	68.4	0	31.6	20	15.03	9.99	74.98
Gentamicin	19	63.2	0	36.8	20	5	5	90
Imipenem	19	68.4	0	31.6	20	9.96	30	59.96
Levofloxacin	15	60	0	40	20	5.04	5.04	89.02
Meropenem	19	68.4	0	31.6	20	14.95	5.02	80.03
Ampicillin / sulbactam	19	68.4	0	31.6	3	100	0	0
Compound sulfamethoxazole	16	56.2	0	43.8	3	100	0	0
Tobramycin	19	47.4	5.3	47.4	18	5.6	0	94.4
Piperacillin / tazobactam	19	57.9	10.5	31.6	19	10.52	21.03	68.45

4. Discussion

Biliary tract infection is one of the common clinical infections, If biliary tract infection is not treated in time, it can often cause local lesions such as liver abscess and systemic lesions such as septic shock [8,9]. Bile duct infections are mostly caused by benign lesions such as bile duct stones, but malignant tumors such as cholangiocarcinoma can also cause bile duct infections. Main causes of biliary infections are poor bile drainage and changes of biliary tract flora. Oddi sphincter which located at the end of bile duct can prevent intestinal bacterial reflux into bile duct effectively, and bile salts can inhibit the growth of bacteria. Therefore, there is no bacterial growth in the bile under physiological condition [10].

The pathogenic bacteria of biliary tract infection mainly come from retrograde infection of gastrointestinal tract. Therefore, the types of pathogenic bacteria of biliary tract infection are closely related to the types of bacteria in the digestive tract. In this study, 693 strains of pathogenic bacteria were isolated and 64.6% were Gram-negative bacteria. There were 210 strains of Escherichia coli (30.3%), 76 strains of Klebsiella pneumoniae (11.0%) and 32 strains of Enterobacter cloacae (4.6%), 35.4% were gram positive bacteria, including 87 strains of Enterococcus faecium (12.6%) and 71 strains of Enterococcus faecalis (10.2%), Similar to the pathogens reported in previous studies, they all belong to digestive tract bacteria [11,12,13]. It can prompt clinicians to use drugs empirically, In the treatment of patients with biliary tract infection, we can improve the function of digestive tract, maintain the patency of gastrointestinal tract and reduce the pressure in gastrointestinal tract., it can also reduce biliary tract infection theoretically [14,15]. However, 3 cases of pathogenic bacteria were Staphylococcus epidermidis, It is considered that the contamination of the sample may be caused by the pathogenic bacteria on the body surface during the operation. So aseptic operation should be strictly carried out.

From the analysis of drug resistance results, Among the pathogens causing biliary tract infection in our hospital, Escherichia coli and Klebsiella pneumoniae had higher resistance rates to penicillins and cephalosporins, while Enterobacter cloacae and Citrobacter had higher resistance rates to cephalosporins. This may be related to the abuse of penicillin antibiotics and cephalosporins, which makes the drug resistance of bacteria continue to improve. The resistance rates of Escherichia coli, Enterobacter cloacae and Citrobacter to carbapenems were low. The resistance rate of Klebsiella pneumoniae to aminoglycoside

antibiotics was low, Aminoglycoside antibiotics have ototoxicity, so clinicians should use them with caution. In recent years, the number of alkanes resistant Enterobacteriaceae in our hospital has increased year by year, which increases the difficulty of clinical treatment.

Enterococcus faecium was resistant to penicillins and quinolones, erythromycin and clindamycin. Vancomycin and linezolid have good antibacterial effect on Enterococcus faecium. Enterococcus faecalis has a high resistance rate to clindamycin and quinupristin / dalfoptin, Penicillin G, ampicillin, linezolid and vancomycin had good antibacterial effect on Enterococcus faecalis. The drug resistance of Enterococcus faecium is more serious than that of Enterococcus faecalis, which is consistent with other scholars[]. For patients with biliary tract infections caused by enterococcus faecium, tetracycline can be given priority for treatment because of the ototoxicity of aminoglycoside antibiotics. For patients with biliary tract infections caused by enterococcus faecalis, penicillin antibiotics are preferred for treatment. The above drugs are ineffective, and vancomycin or linezolid can be used for antibacterial treatment. Among the non-fermenting bacteria, Acinetobacter baumannii has a resistance rate of 68.4% to imipenemm, and the resistance is more serious. Amikacin is recommended, The resistance rate of Pseudomonas aeruginosa to ampicillin / sulbactam and cotrimoxazole was 100%, Pseudomonas aeruginosa has low resistance rates to cephalosporins, hydrocarbonase ene antibiotics, amikacin, cefoperazone/sulbactam, and tobramycin, This kind of medicine can be used in clinic. The detection rate of multi-drug resistant Pseudomonas aeruginosa in our hospital is about 1%, which is still a thorny issue in clinical anti-infection treatment.

In summary, Escherichia coli, Klebsiella pneumoniae and other Enterobacteriaceae were the main pathogens of biliary tract infection in our hospital, The main pathogens of biliary tract infection in our hospital were Escherichia coli, Klebsiella pneumoniae and other Enterobacteriaceae, followed by Enterococcus faecium, Enterococcus faecalis and other enterococci, There are many kinds of drug-resistant bacteria, and the situation of drug resistance is complicated. Clinicians should pay attention to the cultivation of bile specimens and drug susceptibility tests, and obtain reliable dynamic monitoring data of bacterial resistance on a regular basis, so as to effectively guide clinical medication.

Conflicts of Interest

All the authors declared no conflicts of interest.

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