Infection and Drug Resistance

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ORIGINAL RESEARCH

Temporal Shifts in Etiological Agents and Antibiotic Resistance Patterns of Biliary Tract Infections in Sichuan Province, China (2017–2023)

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Purpose: We analyzed the pathogenic bacteria and antibiotic resistance distributions in patients with biliary tract infections (BTI) using samples from the Antimicrobial Resistant Investigation Network of Sichuan Province (ARINSP) to promote the rational use of antibiotics to reduce multidrug resistance.

Patients and Methods: Participating hospitals identified isolates between 2017 and 2023 and conducted antimicrobial susceptibility tests. Isolated bacteria were identified and tested for drug sensitivity using MOLDI-TOF mass spectrometry system, VITEK automated drug sensitivity system and paper diffusion method, and the results were interpreted with reference to CLSI M100 30th edition standards. WHONET 5.6 was used to analyze the results.

Results: In total, 25,573 bacterial isolates were collected; 18,134 were Gram-negative (70.9%). The top five most frequently isolated bacteria were *Escherichia coli* (8,181/25,573; 32.0%), *Klebsiella pneumoniae* (3,247/25,573; 12.7%), *Enterococcus faecium* (2,331/25,573; 9.1%), *Enterococcus faecalis* (1,714/25,573; 6.7%), and *Enterobacter cloacae* (1,429/25,573; 5.6%). *E. coli* and *E. faecalis* slowly declined over time, while *K. pneumoniae* slowly increased; *E. faecium* frequency was stable; *E. coli* resistance to ampicillin was the highest among all antibiotics tested; resistance rates decreased with the addition of sulbactam. *K. pneumoniae* resistance to aztreonam, imipenem, meropenem, ertapenem, and chloramphenicol remained low. *E. cloacae* was highly resistant to cephalosporins, especially cefoxitin and cefazolin. *E. faecalis'* resistance to teicoplanin remained low, decreasing from 6.9% in 2017 to 0.0% in 2019 before stabilizing.

Conclusion: The most frequently isolated bacteria from patients with BTIs were Enterobacteriaceae, including *E. coli* and *K. pneumoniae*, followed by *E. faecium* and *E. faecalis*. Isolates exhibited high resistance to routinely used antibiotics (cephalosporins) and were highly sensitive to tigecycline, carbapenem, amikacin, and vancomycin. The results guide the rational use and continual revision of antibiotic regimens for BTIs to reduce antibiotic resistance.

Keywords: biliary tract infection, bile culture, antimicrobial resistance, microbial profiles

Introduction

The biliary system consists of the intrahepatic and extrahepatic bile ducts.[1] The extrahepatic bile ducts are subdivided into the left, right, and common hepatic ducts, the common bile duct, and the gallbladder; together with the intrahepatic bile ducts, they transport bile secreted by the liver to the duodenal cavity.[2] Physiologic bile production, secretion, storage, exertion, and drainage occur through a sterile biliary system that originates from hepatocytes, ending in the ampulla of Vater in the second portion of the duodenum. The ability of bile to remain sterile directly depends on continuous bile flow in the biliary tree, which includes the intrahepatic system, common hepatic duct, common bile duct, and the gall bladder as a bile reservoir pouch.[3–6] Biliary tract infections (BTI) mainly present as cholecystitis and cholangitis in different parts of the body, categorized as either acute, subacute, or chronic inflammation.[7,8] BTI is primarily caused by biliary

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obstruction and stagnation. Biliary stones are the main cause of obstructions; repeated infections can promote stone formation and further aggravate biliary obstruction.[9] Studies have confirmed that the recurrence and severity of acute cholangitis, along with hospital stay duration, are closely related to biliary bacterial species and drug resistance; compared with patients with non-antimicrobial resistant infections, patients with resistant bacteria have an increased chance of choledocholithiasis recurrence and severe BTI.[5,10]

BTIs are common but have potentially fatal consequences (high rates of late organ failure and mortality) if not recognized and treated promptly. Generally, BTIs are a common cause of bacteremia and are associated with increased mortality rates,[9,10] though with timely and appropriate intervention, mortality can be reduced to <5%.[11] BTI has also been associated with invasive clinical procedures, septicemia, intestinal barrier dysfunction, and translocation of gut bacteria.[12] Antibiotics are key to controlling these infections; however, antibiotic-induced selection pressure can cause overgrowth of pre-existing resistant pathogens in the patient's microbiota, leading to hard-to-treat superinfections.[13] The potential for BTI resistance to antibiotics is high and should be monitored continuously. Understanding the distribution of pathogenic bacteria in BTIs is essential for providing effective care, allowing adjustments to generate more targeted treatments. Incomplete data on the microbial profiles associated with BTI may lead to poor antibiotic therapy and patient prognosis. The spectrum and resistance characteristics of BTIs are constantly changing, and Gram-negative bacilli, some of the most common conditional pathogens in hospitals, have shown increased drug resistance in recent years.

Clinicians who highly suspect BTI usually empirically apply antimicrobial drugs before bacterial culture and drug sensitivity results are available. We conducted this study to understand the characteristics of pathogens causing BTIs and their drug resistance profiles to guide clinical detection and treatment, promote the rational use of antibiotics, and control multidrug-resistant bacterial infections. The importance of a structured, scientific, and individualized approach to antibiotic management in patients with BTIs is evident, but requires insight into the distribution of pathogenic bacteria in patients with BTIs and their resistance trends. To gain this, we retrospectively analyzed data from patients with BTIs in Sichuan Province from 2017 to 2023.

Material and Methods

Bacterial Isolates

The main reference for the diagnosis of biliary tract infections is the Guidelines for the Diagnosis and Treatment of Acute Biliary System Infections (2021 edition). For patients with high suspicion of biliary tract infection, bile was extracted and sent for bacterial culture, and strain identification and antimicrobial susceptibility testing were performed. Bacterial isolates were collected from outpatients and inpatients in ARINSP (Antimicrobial Resistant Investigation Network of Sichuan Province)-participating hospitals from 2017 to 2023; the annual number of hospitals included in the analysis by year was 72 for 2017, 86 for 2018, 92 for 2019, 92 for 2020, 109 for 2022, and 111 for 2023. According to the monitoring protocol, only the first strain of the same bacteria from the same patient was retained. The study was conducted on retrospective data. Ethical approval was obtained from the Institutional Review Board of Sichuan Provincial People's Hospital, and University of Electronic Science and Technology of China (Number: 2021–511).

Identification of Bacterial Species

Identification at the species level was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF) or VITEK-2 automated microbiological analyser (bioMérieux, France).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of isolates was performed using VITEK-2 automated microbiological analyser (bioMérieux, France) to determine minimum inhibitory concentrations. The VITEK-2 system is an automated microbiological identification and drug sensitivity system that enables rapid identification of bacteria and drug sensitivity testing by combining optoelectronic technology, computer technology and bacterial 8-digit identification. Some specific procedures and protocols are as follows: 1) automated operation: the system automatically initialises and controls the temperature of the incubation carousel; 2) card selection: identification and drug susceptibility testing for different

microbial types such as Gram-negative bacteria and Gram-positive bacteria; 3) preparation of standardised bacterial suspensions: using 0.85% NaCl solution and adjusting the concentration of bacterial suspensions in accordance with McFarland's standard; 4) barcode identification: ensure the traceability of the bacterial suspension filling to the card; 5) dynamic monitoring: read the card data at different wavelengths at regular intervals; 6) rapid result interpretation: the endpoint indicator hole reaches the critical value that completes the test, the system automatically compares with the strain database to provide the drug sensitivity results; 7) quality control. Antibiotic discs for Gram-negative microorganisms included ampicillin, amoxicillin with clavulanic acid, cefoperazone with sulbactam, ampicillin with sulbactam, piperacillin with tazobactam, imipenem, ertapenem, meropenem, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefotaxime, cefepime, cefoxitin, amikacin, gentamicin, ciprofloxacin, levofloxacin, sulfamethoxazole, ticarcillin with clavulanic acid, cefotetan, tobramycin, chloramphenicol, tigecycline, polymyxin B, and ceftazidime with avibactam; discs used for Gram-positive isolates were ampicillin, rifampicin, high-concentration gentamicin, high-concentration streptomycin, ciprofloxacin, levofloxacin, linezolid, vancomycin, and ticoranin. To assess sensitivity and resistance according to the antibiogram study, Clinical & Laboratory Standards Institute (CLSI) criteria were followed. Bacterial susceptibility was determined when there was no growth on the edge of the antibiotic disc, while resistant pathogens grew touching the antibiotic disc.

Quality Control

According to the CLSI, a quality control test was performed routinely once a week using *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC27853), and *Escherichia coli* (ATCC 25922) as reference strains.

Statistical Analysis

All data were statistically analysed using WHONET 5.6 software to exclude duplicates. The general steps for WHONET5.6 to perform statistics and analysis of drug sensitivity result data are as follows: 1) Data input: the results of drug sensitivity tests are input into the WHONET5.6 system, including strain information, antimicrobial drug names and corresponding drug sensitivity test results; 2) Selection of analysis type: different types of analyses can be selected in WHONET, such as drug resistance analysis, sensitivity analysis, resistance spectrum analysis, etc.; 3) Setting analysis options: set the corresponding options according to the type of data to be analysed, such as selecting the type of antibiotic of concern and the type of strain; 4) Generating report; 5) Interpretation of results; and 6) Exporting data.

Results

General Distributions and Proportions of Pathogenic Bacteria in Bile Cultures

The distributions and proportions of pathogenic bacteria from bile specimens from 2017 to 2023 are shown in Table 1 and Figure 1. In total, 25,573 clinical bacterial isolates were collected. The strains isolated in each year accounted for 10.0% (2,569/25,573), 10.2% (2,605/25,573), 11.4% (2,926/25,573), 13.7% (3,502/25,573), 15.0% (3,847/25,573), 18.6% (4,746/25,573), and 20.9% (5,357/25,573) of the total isolates, respectively, indicating that the rate of positive bile cultures in patients with BTIs steadily increased over time. The top five most frequently isolated bacteria were *E. coli* (8,181/25,573; 32.0%), *K. pneumoniae* (3,247/25,573; 12.7%), *Enterococcus faecium* (2,331/25,573; 9.1%), *Enterococcus faecalis* (1,714/25,573; 6.7%), and *Enterobacter cloacae* (1,429/25,573; 5.6%); Gram-negative bacterial strains accounted for 70.9% (18,134) of the isolates, and Gram-positive bacterial strains accounted for 29.1% (7,439). Throughout the last six years of data, the ratio of Gram-positive to Gram-negative bacteria was around 3:7. Gram-negative bacteria were mainly *E. coli, K. pneumoniae, E. cloacae*, and *P. aeruginosa*, while Gram-positive bacteria were mainly *E. faecium* and *E. faecalis*. Enterobacteriaceae identified included *E. coli* and *K. pneumoniae*, among others, while non-fermentative bacteria identified included *Acinetobacter baumannii* and *P. aeruginosa*, among others. Data on the primary pathogens across the study period show that the proportion of *E. coli, E. faecium* and *E. faecalis* decreased, whereas that of *K. pneumoniae* and *E. casseliflavus* increased, and *E. cloacae* remained relatively stable (Figure 2).

Table 1 Distribution of Micro-Organisms Isolated from Bile Specimens

	Microbial isolates	2017 (N=2569)		2018 (N=2605)		2019 (N=2926)		2020 (N=3502)		2021 (N=3847)		2022 (N=4767)		2023 (N=5397)	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
Gram-negative bacteria	Escherichia coli	856	47.5	845	46	930	44.9	1112	45.3	1214	44.4	1484	43.8	1740	45.3
	Klebsiella pneumoniae	273	15.1	300	16.3	351	16.9	452	18.4	473	17.3	639	18.9	759	19.8
	Enterobacter cloacae	140	7.8	136	7.4	160	7.7	235	9.6	207	7.6	267	7.9	284	7.4
	Pseudomonas aeruginosa	112	6.2	115	6.3	148	7.1	152	6.2	152	5.6	174	5.1	173	4.5
	Citrobacter freundii	58	3.2	63	3.4	70	3.4	79	3.2	93	3.4	108	3.2	120	3.1
	Aeromonas hydrophila	41	2.3	60	3.3	56	2.7	54	2.2	59	2.2	79	2.3	80	2.1
	Acinetobacter baumannii	52	2.9	47	2.6	47	2.3	30	1.2	53	1.9	75	2.2	75	2.0
	Klebsiella oxytoca	34	1.9	44	2.4	43	2.1	55	2.2	84	3.1	106	3.1	86	2.2
	Others	237	13.2	227	12.4	268	12.9	284	11.6	402	14.7	457	13.5	525	13.6
Gram-positive bacteria	Enterococcus faecium	242	31.6	248	32.3	276	32.4	327	31.2	359	32.3	421	30.6	458	30.2
	Enterococcus faecalis	233	30.4	172	22.4	224	26.3	238	22.7	240	21.6	298	21.6	309	20.4
	Enterococcus casseliflavus	42	5.5	64	8.3	64	7.5	103	9.8	114	10.3	123	8.9	135	8.9
	Enterococcus gallinarum	49	6.4	60	7.8	59	6.9	67	6.4	76	6.8	82	6.0	106	7.0
	Others	200	26.1	224	29.2	230	27.0	314	30.0	321	28.8	454	33.0	507	33.4

Notes: N, the annual total number of microbial isolates; n, the actual number of each bacteria.

Analysis of Gram-Negative Pathogenic Bacteria Drug Resistance Rates

The specific drug resistance rates for *E. coli, K. pneumonia, E. cloacae*, and *P. aeruginosa* isolates are shown in Tables 2–5, respectively. *E. coli* was generally highly resistant to penicillins, cephalosporins, and quinolones. Among all the antimicrobials tested, *E. coli* had the highest resistance to ampicillin (remaining around 78.0%); although the rate of resistance decreased after the combination of ampicillin and sulbactam, it remained high (~50.1%). *E. coli* was more susceptible to cefoperazone-sulbactam and piperacillin-tazobactam (90.8% and 91.3% susceptible, respectively). Resistance rates of *E. coli* to carbapenems (imipenem, meropenem, and ertapenem) were extremely low, at 1.1%, 1.1%, and 1.6%, respectively. *E. coli* was more resistant to ciprofloxacin and levofloxacin (47.8% and 43.3%, respectively), and more sensitive to amikacin (1.7%).

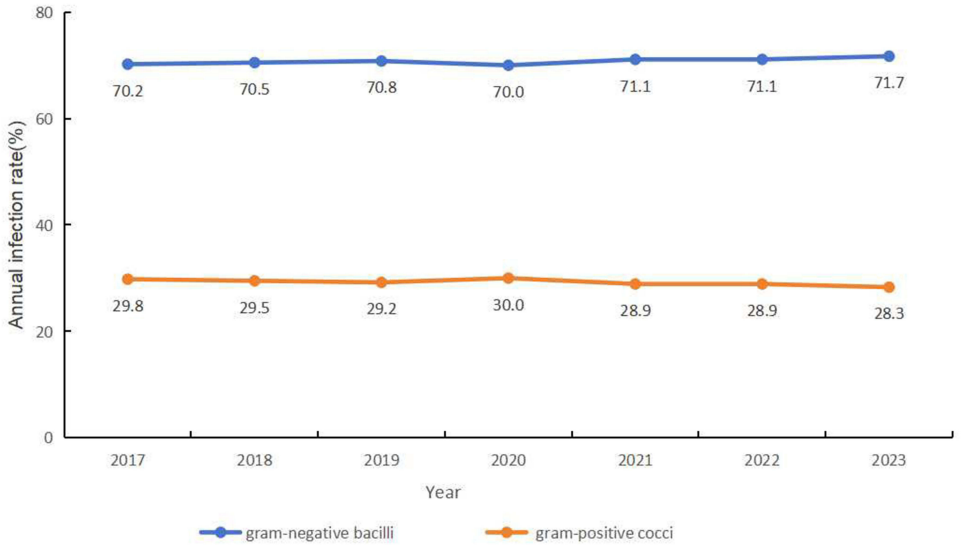

Figure 1 Changes in the positive rate of gram-negative bacilli and gram-positive cocci.

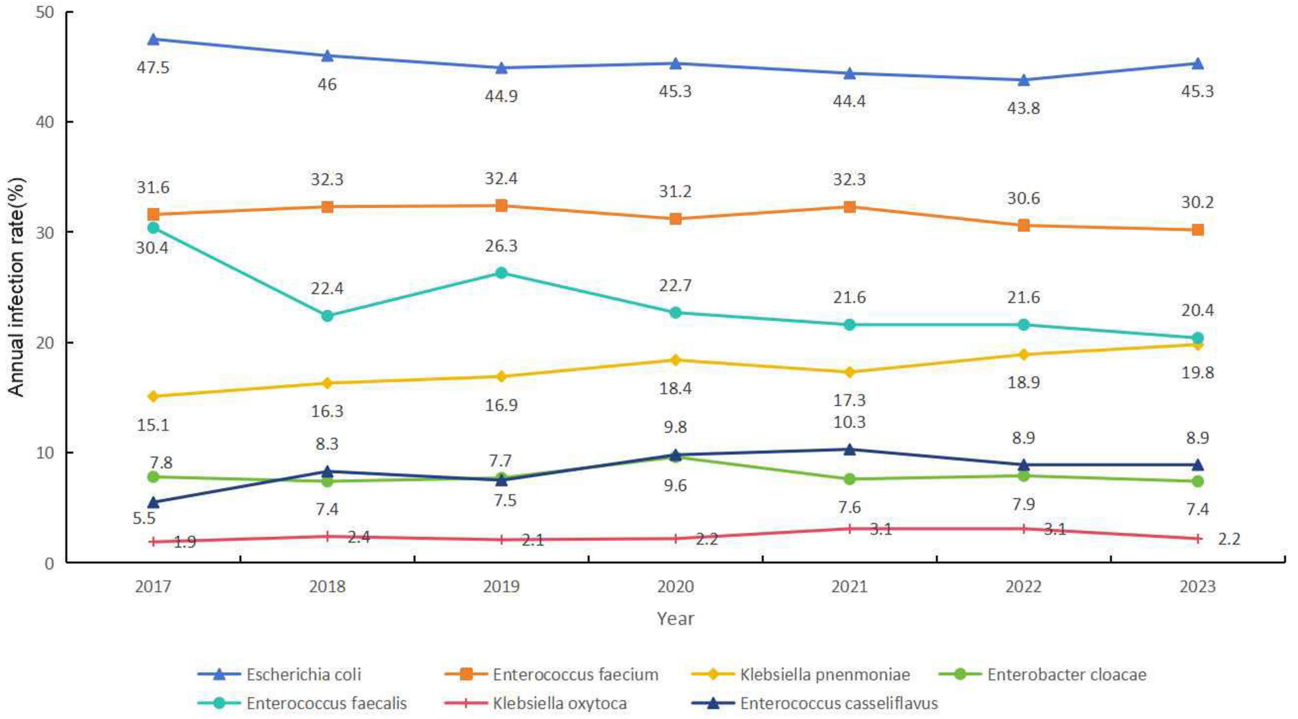

Figure 2 Trend change in the percentage of pathogenic bacteria detected in bile culture.

Similar to *E. coli, K. pneumoniae* was more susceptible to cefoperazone-sulbactam (91.7%) and piperacillin-avibactam (88.7%) and was highly resistant to cephalosporins, except for cefotetan (7.3% in 2018, 7.8% in 2020, and 7.6% in 2021). The resistance of *K. pneumoniae* to meropenem (1.6% in 2017, 6.4% in 2018, 2.1% in 2019, 1.5% in 2020, 4.3% in 2021, 4.4% in 2022, and 3.0% in 2023), imipenem (3.3% in 2017, 3.4% in 2018, 1.7% in 2019, 2.7% in 2020, 4.4% in 2021, 3.6% in 2022, and 2.6% in 2023), and ertapenem (0.8% in 2017, 5.5% in 2018, 3.1% in 2020, and 4.0% in 2021) remained low; its susceptibility to all three of these carbapenems fluctuated highly, but there was a slow rise in meropenem resistance.

Table 2 Antibiotic Resistance of Escherichia Coli

Antibiotics	2017 (N=856)		2018 (N=845)		2019 (N=930)		2020 (N=1112)		2021 (N=1214)		2022 (N=1484)		2023 (N=1740)	
	n	R%	n	R%	n	R%	n	R%	n	R%	n	R%	n	R%
Ampicillin	743	77.8	816	77.7	925	73.4	1029	79.6	1033	81.3	1226	79.0	1522	77.3
Amoxicillin/clavulanate	338	18.3	77	19.5	166	27.1	335	24.8	616	28.2	776	20.7	1122	23.0
Cefoperazone/sulbactam	222	8.1	235	8.5	268	9.0	497	9.9	751	9.7	936	10.7	1195	8.7
Ampicillin/sulbactam	526	52.9	741	51.8	889	48.9	1013	51.8	1104	53.4	1282	48.9	1595	43.2
Piperacillin/tazobactam	791	7.0	823	6.0	890	6.0	1105	8.2	1200	8.2	1286	13.8	1574	11.7
Cefazolin	298	67.8	399	67.2	488	56.6	661	68.8	758	67.9	922	63.3	1145	63.8
Cefuroxime	357	54.1	332	53.0	552	48.9	695	59.3	818	56.5	1033	56.3	1274	54.2
Ceftazidime	778	31.0	780	27.2	901	29.6	1109	33.6	1205	31.4	1381	30.2	1716	28.0
Ceftriaxone	661	50.8	801	49.3	886	45.3	1101	52.9	1193	51.5	1291	51.8	1509	50.7
Cefotaxime	198	51.0	76	52.6	299	43.8	482	54.1	560	50.7	685	53.6	846	52.1
Cefepime	724	27.1	708	22.6	863	20.6	1043	23.6	1128	23.6	1322	20.9	1599	21.8
Cefoxitin	336	17.9	270	14.4	300	18.3	362	13.3	571	16.8	510	13.3	563	13.5
Aztreonam	596	38.4	674	38.9	801	35.3	946	40.2	1037	41.1	1221	37.2	1550	37.2
Ertapenem	483	0.8	588	1.9	–	–	931	1.7	983	1.9	–	–	–	–

(*Continued*)

Table 2 (Continued).

Antibiotics	2017 (N=856)		2018 (N=845)		2019 (N=930)		2020 (N=1112)		2021 (N=1214)		2022 (N=1484)		2023 (N=1740)	
	n	R%	n	R%	n	R%	n	R%	n	R%	n	R%	n	R%
Imipenem	845	0.5	834	1.2	916	0.8	1104	1.4	1167	1.5	1349	1.3	1698	0.9
Meropenem	370	0.5	344	2.3	514	0.4	634	1.3	888	1.2	933	1	1227	1.1
Amikacin	791	1.9	783	1.3	877	2.4	1071	1.8	1203	2.1	1351	1.2	1680	1.1
Gentamicin	816	25.1	839	25.6	923	25.6	1006	24.3	1106	22.2	1289	21.9	1572	22.5
Ciprofloxacin	733	50.3	816	45.3	895	45.5	860	61.3	1082	48.1	1257	40.7	1470	43.3
Levofloxacin	757	43.2	754	41.8	857	41.5	931	51.3	1128	45.6	1405	40.1	1693	39.9
Sulfamethoxazole	796	42.2	821	40.0	869	39.6	1085	44.9	1142	43.9	1273	41	1583	42.0
Ticarcillin/clavulanate	–	–	84	28.6	–	–	271	25.8	348	27.6	–	–	–	–
Cefotetan	–	–	485	5.4	–	–	661	4.4	644	5.9	–	–	–	–
Tobramycin	–	–	611	11.9	–	–	896	11.4	942	11.8	–	–	–	–
Chloromycetin	–	–	88	23.9	97	24.7	141	47.5	256	36.7	185	40	239	36.4
Tigecycline	–	–	91	0	267	0	439	0	602	0.2	740	0	956	0.1
Polymyxin B	–	–	–	–	–	–	–	–	16	0	–	–	–	–
Ceftazidime/avibactam	–	–	–	–	–	–	–	–	74	14.9	–	–	–	–

Notes: N, the annual total number of Escherichia coli; n, the actual number of each antibiotic testing susceptibility, R%, the resistance rates of Escherichia coli to each antibiotic; -, not applicable or not tested.

Table 3 Antibiotic Resistance of Klebsiella Pneumoniae

Antibiotics	2017 (N=273)		2018 (N=300)		2019 (N=351)		2020 (N=452)		2021 (N=473)		2022 (N=639)		2023 (N=759)	
	n	R%	n	R%	n	R%	n	R%	n	R%	n	R%	n	R%
Amoxicillin/clavulanate	125	20.8	30	6.7	57	10.5	146	22.6	242	15.3	348	16.1	476	14.9
Cefoperazone/sulbactam	85	8.2	104	7.7	101	5.9	207	9.2	274	8.4	405	9.1	511	9.6
Ampicillin/sulbactam	178	37.1	264	33.3	333	32.4	421	33.5	439	36.9	541	35.1	691	28.5
Piperacillin/tazobactam	265	11.3	299	8.7	330	7.9	449	10.0	467	12.2	567	14.5	694	14.3
Cefazolin	96	45.8	134	36.6	206	39.8	244	35.2	283	41.7	365	35.9	471	33.3
Cefuroxime	126	29.4	134	26.9	220	36.8	257	30.4	317	37.5	453	28.7	562	32.7
Ceftazidime	245	18.0	286	16.1	333	19.2	451	17.5	468	21.6	596	19.1	751	17.6
Ceftriaxone	213	29.6	274	21.9	327	24.5	448	23.2	462	31.0	568	26.6	684	26.2
Cefotaxime	78	26.9	39	33.3	119	32.8	194	23.2	206	30.6	288	26.7	381	24.1
Cefepime	234	16.7	251	11.2	327	10.1	435	12.6	455	13.2	584	12.2	708	11.9
Cefoxitin	129	17.8	123	14.6	109	21.1	147	17.7	202	18.8	247	13.4	288	12.8
Aztreonam	199	25.6	240	20.0	303	20.8	382	20.7	405	24.7	519	23.5	667	22.2
Ertapenem	483	0.8	182	5.5	–	–	357	3.1	375	4.0	–	–		
Imipenem	271	3.3	295	3.4	349	1.7	448	2.7	455	4.4	577	3.6	739	2.6
Meropenem	127	1.6	141	6.4	188	2.1	270	1.5	328	4.3	408	4.4	541	3.0
Amikacin	265	3.4	293	3.1	331	1.8	431	1.6	470	3.6	581	2.8	735	1.9
Gentamicin	262	17.9	299	11.4	346	14.2	407	11.5	431	13.2	539	12.8	697	11.0
Ciprofloxacin	230	25.7	289	18.7	339	20.4	338	36.1	435	22.5	517	19.3	611	16.7
Levofloxacin	259	20.1	275	15.6	318	15.7	364	25.0	437	18.8	596	15.6	728	14.1
Sulfamethoxazole	256	28.5	292	23.3	330	25.8	433	26.8	449	23.4	555	23.4	692	20.8
ticarcillin/clavulanate	–	–	40	17.5	–	–	125	17.6	141	21.3	–	–	–	–
Cefotetan	–	–	143	4.9	–	–	263	4.6	258	8.9	–	–	–	–
Tobramycin	–	–	218	7.3	–	–	358	7.8	370	7.6	–	–	–	–

(*Continued*)

Table 3 (Continued).

Antibiotics	2017 (N=273)		2018 (N=300)		2019 (N=351)		2020 (N=452)		2021 (N=473)		2022 (N=639)		2023 (N=759)	
	n	R%	n	R%	n	R%	n	R%	n	R%	n	R%	n	R%
Chloromycetin	–	–	46	43.5	36	27.8	59	28.8	96	31.3	98	33.7	128	28.1
Tigecycline	–	–	48	2.1	108	2.8	177	1.1	199	2.0	288	2.1	417	2.4
Polymyxin B	–	–	–	–	–	–	–	–	5	0.0	–	–	–	–
Ceftazidime/avibactam	–	–	–	–	–	–	–	–	32	9.4	–	–	–	–

Notes: N, the annual total number of Klebsiella pneumoniae; n, the actual number of each antibiotic testing susceptibility, R%, the resistance rates of Klebsiella pneumoniae to each antibiotic; -, not applicable or not tested.

Table 4 Antibiotic Resistance of Enterobacter Cloacae

Antibiotics	2017 (N=140)		2018 (N=136)		2019 (N=160)		2020 (N=235)		2021 (N=207)		2022 (N=267)		2023 (N=284)	
	n	R%	n	R%	n	R%	n	R%	n	R%	n	R%	n	R%
Cefoperazone/sulbactam	31	16.1	30	13.3	52	13.5	89	11.2	120	10.8	151	7.9	147	11.6
Piperacillin/tazobactam	126	22.2	131	13	152	15.8	233	16.7	203	22.2	231	39.4	259	33.6
Ceftazidime	126	46	130	43.8	148	41.2	234	40.2	205	45.4	250	46.8	280	43.9
Ceftriaxone	109	53.2	129	52.7	154	46.8	227	44.1	205	50.2	236	53.4	239	47.3
Cefotaxime	28	46.4	15	66.7	48	45.8	105	48.6	85	57.6	110	59.1	151	48.3
Cefepime	115	7	110	11.8	152	8.6	220	5.5	199	5	243	5.8	265	6.4
Aztreonam	103	48.5	108	44.4	133	42.1	192	40.1	164	48.8	213	45.5	257	39.7
Ertapenem	56	1.8	70	12.9	–	–	160	5.6	129	9.3	–	–	–	–
Imipenem	136	3.7	126	4	155	1.9	230	1.7	198	1	243	4.1	277	2.2
Meropenem	65	3.1	67	4.5	91	7.7	127	2.4	139	1.4	165	3.6	198	2.5
Amikacin	139	0.7	136	1.5	149	0.7	204	0	207	0	247	0.4	275	1.5
Gentamicin	138	2.2	136	3.7	156	5.1	216	2.3	193	3.1	230	3.9	260	2.3
Ciprofloxacin	124	14.5	126	12.7	154	11	169	17.2	192	7.8	221	6.8	244	6.1
Levofloxacin	128	10.9	126	7.1	150	2.7	174	10.9	194	6.2	257	4.7	273	4.8
Sulfamethoxazole	132	7.6	134	9	146	8.9	218	8.3	187	10.7	226	6.6	250	8.0
Ampicillin	–	–	54	88.9	–	–	76	88.2	74	87.8	–	–	–	–
amoxicillin/clavulanate	–	–	9	77.8	–	–	86	93	80	92.5	–	–	–	–
ampicillin/sulbactam	–	–	49	75.5	–	–	87	79.3	78	73.1	–	–	–	–
ticarcillin/clavulanate	–	–	21	14.3	–	–	47	38.3	68	52.9	–	–	–	–
cefazolin	–	–	99	99	–	–	156	100	145	99.3	–	–	–	–
cefuroxime	–	–	30	76.7	–	–	108	72.2	101	80.2	–	–	–	–
cefotetan	–	–	51	54.9	–	–	85	60	85	67.1	–	–	–	–
cefoxitin	–	–	38	94.7	–	–	72	97.2	52	100	–	–	–	–
tobramycin	–	–	93	5.4	–	–	188	0.5	162	3.1	–	–	–	–
chloromycetin	–	–	22	18.2	–	–	26	19.2	35	20	–	–	–	–
tigecycline	–	–	26	0	57	0	90	1.1	92	0	139	0	144	1.4
polymyxin B	–	–	–	–	–	–	–	–	1	100	–	–	–	–
ceftazidime/avibactam	–	–	–	–	–	–	–	–	18	55.6	–	–	–	–

Notes: N, the annual total number of Enterobacter cloacae; n, the actual number of each antibiotic testing susceptibility, R%, the resistance rates of Enterobacter cloacae to each antibiotic; -, not applicable or not tested.

E. cloacae was highly resistant to cephalosporins, except for cefepime. Resistance of *E. cloacae* to quinolones fluctuated at low levels. Although amikacin (0.7%) and gentamicin (3.2%) are both aminoglycosides, they were more sensitive to amikacin. Increased ticarcillin/clavulanate was seen, from 14.3% in 2018 and 38.3% in 2020 to 52.9% in 2021; an increase in amoxicillin/clavulanate was also seen, from 77.8% in 2018 to 93% in 2020 to 92.5% in 2021. The resistance of *E. cloacae* to

Table 5 Antibiotic Resistance of *Pseudomonas aeruginosa*

Antibiotics	2017 (N=112)		2018 (N=115)		2019 (N=148)		2020 (N=152)		2021 (N=152)		2022 (N=174)		2023 (N=173)	
	N	R (%)	N	R (%)	N	R (%)	N	R (%)	N	R (%)	n	R (%)	N	R(%)
Piperacillin	30	26.7	26	26.9	39	25.6	64	21.9	86	17.4	88	30.7	77	33.8
Cefoperazone/sulbactam	20	5.0	42	16.7	44	13.6	71	12.7	71	14.1	84	11.9	100	10.0
Piperacillin/tazobactam	98	11.2	108	11.1	142	16.9	149	12.8	150	16	157	17.2	162	14.2
Ceftazidime	94	18.1	109	16.5	143	17.5	151	13.2	152	18.4	158	23.4	170	21.2
Cefepime	106	11.3	111	9.9	145	14.5	150	7.3	145	13.8	159	15.7	166	8.4
Aztreonam	63	30.2	72	27.8	72	20.8	83	24.1	121	12.4	116	21.6	112	24.1
Imipenem	102	21.6	101	15.8	144	19.4	151	15.2	144	16	155	18.7	158	15.8
Meropenem	38	15.8	69	11.6	72	15.3	83	18.1	123	10.6	121	14.9	135	12.6
Amikacin	105	0.0	111	0.9	129	1.6	121	1.7	152	0	156	1.3	167	0.6
Gentamicin	110	11.8	114	8.8	124	7.3	143	2.8	147	4.8	152	7.9	141	7.8
Tobramycin	103	8.7	97	5.2	119	5.9	137	3.6	144	4.2	158	4.4	168	4.8
Ciprofloxacin	100	20	95	9.5	127	16.5	131	11.5	148	6.8	159	11.3	165	10.3
Levofloxacin	99	15.2	109	17.4	121	12.4	103	18.4	143	9.1	157	11.5	154	19.5
Polymyxin B	5	0.0	7	14.3	–	–	16	0	15	6.7	–	–	–	–
Ceftazidime/avibactam	–	–	–	–	–	–	–	–	2	50	–	–	–	–

Notes: N, the annual total number of *Pseudomonas aeruginosa*; n, the actual number of each antibiotic testing susceptibility, R%, the resistance rates of *Pseudomonas aeruginosa* to each antibiotic; -, not applicable or not tested.

ampicillin (88.9% in 2018, 88.2% in 2020, and 87.8% in 2021) declined slightly over time but remained high; similarly, its resistance to carbapenems fluctuates at lower levels.

P. aeruginosa resistance to amikacin remained low. The resistance of *P. aeruginosa* to amikacin (0.0% in 2017, 0.9% in 2018, 1.6% in 2019, 1.7% in 2020, 0.0% in 2021, 1.3% in 2022, and 0.6% in 2023) remained low, while imipenem and meropenem resistance rates fluctuated around 17.8% and 14.4%, respectively.

Analysis of Gram-Positive Pathogenic Bacteria Drug Resistance Rates

The specific drug resistance rates for Gram-positive pathogenic bacteria in the bile of patients with biliary tract infections are shown in Table 6 and Table 7. Both Enterococcus faecium and *Enterococcus faecalis* showed significantly reduced resistance to high concentrations of gentamicin and streptomycin. The linezolid resistance level of Enterococcus faecium remained the lowest among all antibiotics tested (0.0% from 2017 to 2021) but increased to 1.4% in 2023. Compared with the resistance rates of *Enterococcus faecalis* to ciprofloxacin (8.1%) and levofloxacin (7.2%), Enterococcus faecium

Table 6 Antibiotic Resistance of Enterococcus Faecium

Antibiotics	2017 (N=242)		2018 (N=248)		2019 (N=276)		2020 (N=327)		2021 (N=359)		2022 (N=421)		2023 (N=	
	N	R (%)	N	R (%)	N	R (%)	N	R (%)	N	R (%)	N	R (%)	N	R(%)
Ampicillin	231	51.1	242	48.8	271	50.9	323	42.1	358	41.1	392	46.7	449	25.9
High concentration of gentamicin	178	33.1	200	27.5	231	25.5	265	22.3	319	22.9	332	19.3	400	16.2
High concentration of streptomycin	172	29.7	176	22.2	200	15.5	251	20.3	302	17.2	328	11.6	389	14.7
Rifampicin	67	50.7	88	72.7	100	67	123	55.3	118	55.9	121	63.6	76	44.7
Ciprofloxacin	205	51.2	217	51.6	226	53.5	274	43.4	289	47.1	305	50.8	371	48.2
Levofloxacin	207	44.0	205	44.4	242	51.7	279	38.4	311	43.1	367	45.8	408	43.6
Linezolid	209	0.0	213	0.0	231	0.0	303	0.0	348	0.0	381	0.3	437	1.4
Vancomycin	235	0.4	239	1.3	276	0.4	327	0.0	356	0.0	392	0.5	452	0.9
Ticoranin	33	0.0	27	3.7	107	0.9	107	0.9	116	0.0	74	1.4	63	3.2

Notes: N, the annual total number of Enterococcus faecium; n, the actual number of each antibiotic testing susceptibility, R%, the resistance rates of Enterococcus faecium to each antibiotic;

Table 7 Antibiotic Resistance of *Enterococcus faecalis*

Antibiotics	2017 (N=233)		2018 (N=172)		2019 (N=224)		2020 (N=238)		2021 (N=240)		2022 (N=298)		2023 (N=309)	
	N	R (%)	N	R (%)	N	R (%)	N	R (%)	N	R (%)	N	R (%)	N	R(%)
Ampicillin	225	1.8	155	1.9	213	4.2	238	2.9	238	3.4	280	3.6	301	3.3
High concentration of gentamicin	152	18.4	126	8.7	171	11.7	196	9.7	189	9.5	233	8.6	268	11.2
High concentration of streptomycin	138	18.8	113	15.9	166	16.3	184	12	183	15.3	228	9.6	236	12.3
Rifampicin	78	32.1	71	33.8	89	36	75	36	52	38.5	51	37.3	44	29.5
Ciprofloxacin	197	7.1	136	9.6	187	7.5	209	6.7	189	7.9	238	6.7	231	11.3
Levofloxacin	202	3.5	137	8.8	180	5	213	7	213	7	273	9.9	278	9.4
Linezolid	182	1.1	131	1.5	177	0.6	193	1	212	0.5	261	1.1	283	0.7
Vancomycin	214	0.5	164	1.2	218	0	235	0.4	238	0.8	279	0	303	0
Ticoranin	29	6.9	35	2.9	96	0	66	0	55	0	32	0	44	0

Notes: N, the annual total number of *Enterococcus faecalis*; n, the actual number of each antibiotic testing susceptibility, R%, the resistance rates of *Enterococcus faecalis* to each antibiotic;

had a higher rate of resistance (49.4% and 44.4%, respectively). However, an increase in *Enterococcus faecalis* resistance to levofloxacin was seen (3.5% in 2017, 8.8% in 2018, 5.0% in 2019, 7.0% in 2020, 7.0% in 2021, 9.9% in 2022, and 9.4% in 2023). The susceptibility of Enterococcus faecium and *Enterococcus faecalis* to vancomycin and ticlopidine fluctuated highly, and the resistance of *Enterococcus faecalis* to ticlopidine remained at 0 for the last five years. The level of resistance of *Enterococcus faecalis* to linezolid remained relatively stable over the six years examined (1.1% in 2017, 1.5% in 2018, 0.6% in 2019, 1.0% in 2020, 0.5% in 2021, 1.1% in 2022, and 0.7% in 2023).

Discussion

BTI is a common disease with different clinical manifestations. Its main cause is bile duct obstruction caused by gallstones, especially with choledocholithiasis, which increases pressure in the bile ducts and leads to microbial colonization and proliferation.[14] Timely use of antimicrobial drugs to control symptoms and prevent further pathogen spread is key to effective treatment. This study analyzed the distribution and resistance of pathogenic microorganisms in the bile of patients with BTIs.

Assessing microbial profiles and antibiotic susceptibility patterns in bile cultures can help guide effective empirical antibiotic therapy. In the present study, the annual total number of collected strains increased over time. Gram-negative bacteria were dominant (70.2% in 2017, 70.5% in 2018, 70.8% in 2019, 70.0% in 2020, 71.1% in 2021, 71.1% in 2022, and 71.7% in 2023) and slightly increased in detection frequency during the study period. Of all the isolates during the study period, Gram-negative bacilli (70.8%) were dominant, followed by Gram-positive cocci (29.2%), which was also seen in most previous studies.[6,10,12,15,16] In a study examining bile from patients with suspected acute cholangitis, Gromski et al found that pathogens detected included *Enterococcus* spp. (67.6%), *Klebsiella* spp. (44.5%), *E. coli* (40.6%), *Pseudomonas* spp. (7.8%), and anaerobes (9.6%).[11] A prospective study in a Spanish hospital found that the most frequently isolated facultative microorganisms were *Enterococcus* spp., which were present in 16 out of the 44 (36.7%) patients with bactobilia; this was followed by *E. coli* and *Klebsiella* spp.[4] The top five most frequently isolated bacteria in the present study were *E. coli, K. pneumoniae, E. faecium, E. faecalis*, and *E. cloacae*. The distribution of common pathogens among patients with BTIs may differ significantly in different countries or regions. Similar to most previous studies, *E. coli* and *K. pneumoniae* were the most common microorganisms isolated from patients with BTIs;[4,6,14,17] however, their isolation frequencies changed slightly over time during the study period. *E. coli* prevalence declined slowly, but that of *K. pneumoniae* slowly increased. The detection frequency of *E. faecium* remained relatively stable over the study period, while that of *E. faecalis* declined gradually. These results differ from the findings of Zhao et al in 2019, who found that the detection frequencies of *E. coli* and *K. pneumoniae* slowly declined over time, but those of *E. faecium* and *E. faecalis* gradually increased in recent years.[14]

Detection of pathogen resistance is a vital reference for guiding clinical rational drug use; local patterns of antibiotic resistance can be used as supporting data to optimize the selection of empirical antibiotic therapy and increase the appropriate use of antibiotic drugs, reducing mortality and healthcare costs. In the present study, *E. coli* and *K. pneumoniae* isolates were resistant to cefuroxime (54.6% and 31.8%, respectively) and aztreonam (38.3% and 22.5%, respectively), and had high resistance to the first-/third-generation cephalosporins (>30% and >20%, respectively). Similar to the findings of Zhao et al, *E. coli* had the highest resistance to ampicillin (remaining around 78.0%); although its rate of resistance decreased after the combination of ampicillin and sulbactam, it remained high (around 50.1%).[14] Both bacteria remained highly susceptible to cefotetan, carbapenems, and tigecycline, as well as to quinolones (especially levofloxacin); however, other members of our group have identified an increasing trend of resistance to carbapenem antibiotics in *K. pneumoniae*, while tigecycline remains effective.[18] These results indicate that the conditions for the use of carbapenems in patients with BTIs should be strictly controlled. Aminoglycosides are used less in the clinic because of their side effects, whereas quinolones are commonly used to treat respiratory, gastrointestinal, and urinary tract infections. *E. coli* was more resistant to ciprofloxacin and levofloxacin (47.8% and 43.3%, respectively), but more sensitive to amikacin (1.7%), supporting the widespread use of amikacin in clinical practice. *K. pneumoniae* is the most common pathogen causing neonatal infections, leading to high mortality worldwide. Along with increasing antimicrobial use in neonates, carbapenem-resistant *K. pneumoniae* (CRKP) has emerged as a severe challenge for infection control and treatment.[19] As the resistance mechanisms of *K. pneumoniae* may vary in different populations and regions, future surveillance is crucial.[20]

All five common Gram-negative bacteria had relatively high susceptibility to combinations of two antimicrobial drugs, such as cefoperazone-sulbactam and piperacillin-tazobactam. Though the results of drug susceptibility testing for ceftazidime-avibactam, a new antimicrobial drug that only became available in 2015, are incomplete, its availability provides a new therapeutic option for many serious and difficult-to-treat infections.[21] Polymyxins are a last-line defense against difficult-to-treat multi-drug resistance Gram-negative pathogens; however, the emergence and prevalence of polymyxin-resistant bacteria have been detected over the last several years. If resistance is allowed to develop further, there will be a lack of drugs available for treating infections.[8,22] This means that optimizing its clinical application and discovering next-generation polymyxins are crucial for future treatment efficacy.[23]

Enterococci were the main Gram-positive pathogens causing BTIs in the present study. Previous research has shown that among Gram-positive bacteria, *E. faecalis* is one of the most common causes of bacteremia.[10] Biliary-tract bloodstream infections caused by *E. faecalis* and *E. faecium* are associated with inappropriate empirical treatment and worse outcomes.[24] Choosing the most appropriate empirical antimicrobial treatment is crucial for treatment efficacy and improved prognosis of BTIs. Compared with *E. faecalis, E. faecium* had higher resistance to ampicillin, rifampicin, and fluoroquinolones. Almost all *E. faecium* and *E. faecalis* isolates were susceptible to vancomycin, ticlopidine, and linezolid. For BTIs caused by Enterococci, vancomycin is the drug of choice for empirical therapy; the Tokyo guidelines also recommend vancomycin for grade III cases.[16] The resistance of *E. faecium* and *E. faecalis* to high concentrations of gentamicin and streptomycin gradually decreased during the study period; however, given the side effects of highly concentrated antimicrobials, clinicians rarely use them.

A study from the Binhai Bay Central Hospital in Dongguan City, Guangdong Province, found an increase in resistance to ciprofloxacin and a decrease in resistance to amikacin in *Escherichia coli*, an increase in resistance to cephalosporins, carbapenems, β-lactam inhibitors, aminoglycosides, and quinolones in *Klebsiella pneumoniae*, and a significant decrease in resistance to certain drugs in *Pseudomonas aeruginosa*.[25] A study at the Affiliated Hospital of Qingdao University showed that enterococci and *Escherichia coli* showed high resistance to conventional antimicrobials but remained highly susceptible to piperacillin, tazolol, bactam carbon, penicillins, amikacin and vancomycin.[7] It is amply demonstrated that pathogenic microbial species and antimicrobial drug susceptibility of BTI vary from one region to another or at different time stages in the same region.

In addition, we found that combination antibiotic therapies such as ampicillin-sulbactam, piperacillin-tazobactam, and cefoperazone-sulbactam reduced antibiotic resistance compared with the use of a single antibiotic; these are combinations of currently commonly used beta-lactams with resistance enzyme inhibitors. As the present study and several similar reports have shown, although the distribution of biliary bacteria did not change significantly over time, signs of

resistance to currently recommended antibiotics emerged.[6,14,26] Considering this, it is strongly recommended that similar long-term multicenter studies be conducted to carefully monitor changes in bacterial development, especially in the context of antibiotic resistance, and that relevant antimicrobial usage guidelines be revised.

Monitoring changes in pathogenic microorganisms and antibiotic resistance in patients with biliary tract infections is of great clinical significance in guiding clinical treatment and improving patient prognosis. First, accurate monitoring of pathogenic microorganisms can help to clarify the type and severity of infection, and provide targeted treatment plans for the clinic. Secondly, antibiotic resistance monitoring can reflect the trend of drug resistance in time, guide doctors to choose antibacterial drugs reasonably, avoid inappropriate antibiotic use, and reduce the production and spread of drug-resistant strains. In addition, imbalances in the biliary microbiota may also contribute to the development and progression of biliary diseases; therefore, monitoring of the biliary microbiota is equally important for the prevention, diagnosis and treatment of biliary diseases.[27] A study have shown that the composition of the gut microbiota in patients with BTI is significantly different from that of the healthy population.[28] Then, we can detect the changes of gut microbiota in patients with suspected biliary tract infections to clarify the diagnosis and prevent the use of antimicrobial drugs in advance, which is of great significance for the diagnosis, treatment and prognosis of patients with BTI.

Despite its findings, some limitations of the present study should be noted. Antibiotic exposure may affect positive bile cultures and microbiologic profiles; as patient information was not available, we did not analyze antimicrobial resistance rates in outpatients and inpatients or determine what antimicrobial patients had previously used. Patients with different comorbidities such as diabetes mellitus, hypertension, coronary artery disease, and immunosuppression are also at increased risk for biliary tract infections, but these factors mainly affect the dose and frequency of antimicrobials, and the choice of antimicrobials is mainly a matter of localised past bacterial infections and resistance patterns and final bile cultures and sensitisation results. Additionally, not all hospitals met the criteria (lacking personnel, devices, facilities, and/or methods) to participate in the program to ensure monitoring accuracy, meaning we were unable to capture information for all BTIs. Different hospitals also do not use exactly the same testing methods; some use inconsistent methods that may have affected the results. Finally, as microbial frequencies and antimicrobial resistance patterns have some geographic and regional differences, this may constrain the generalizability of this study.

Conclusion

The bacteria most commonly isolated from positive bile cultures in patients with BTIs were Enterobacteriaceae, including *E. coli* and *K. pneumoniae*, followed by Enterococci including *E. faecium* and *E. faecalis*. These primary bacterial isolates exhibited high resistance to routinely used antibiotics (such as cephalosporins) but were highly sensitive to tigecycline, carbapenem, amikacin, and vancomycin. When clinicians have a high suspicion that a patient has a BTI, antimicrobials that cover both Gram-negative bacilli and Gram-positive cocci with high bile penetration, such as tigecycline, cefoperazone/sulbactam, and piperacillin/tazobactam, should be used; patients should be closely monitored and antimicrobials adjusted in a timely manner as bile culture and drug susceptibility results become available. As the distribution and resistance profiles of pathogenic organisms in patients with BTIs may vary in different populations and regions, proactive measures to reduce antibiotic resistance and real-time monitoring are essential. What measures can reduce antibiotic resistance? For example, 1) standardising antibiotic use, 2) timely sampling and collection of bile specimens before starting antimicrobials; 3) choosing appropriate antimicrobials, 4) following discontinuation indications and dosing regimens to reduce antibiotic pressure; and 5) multidisciplinary collaborative treatment to promote multidisciplinary teamwork and more standardised use of antibiotics in complex cases. Surveillance can help us to continually revise existing empirical antibiotic regimens for BTIs, reduce antibiotic resistance, and provide more rational and scientific guidelines for the use of antimicrobials.

Abbreviations

BTI, biliary tract infection; CLSI, Clinical and Laboratory Standards Institute; ARINSP, Antimicrobial Resistant Investigation Network of Sichuan Province.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work. The data and samples accessed comply with the relevant data protection and privacy regulations.

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