Original Article

Distribution analysis of positive and negative pathogenic bacteria in patients with acute pancreatitis and the clinical characteristics and model prediction analysis of positive infection bacteria

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Background: There were bacteria in the early pancreatic juice culture of severe acute pancreatitis (SAP) patients, but during the clinical time, some patients showed more positive bacteria and some patients showed more negative bacteria. Many scholars have different test results, and further clinical research needs to be carried out to clarify this fact. To determine evidence of infection in the early stage of acute pancreatitis (AP) by pancreatic juice bacterial culture and provide a reference for the anti-infective therapy of AP.

Methods: Patients with AP who underwent pancreatic juice bacterial culture in the Department of hepatobiliary surgery of the General Hospital of Ningxia Medical University from January 1, 2019 to June 30, 2020were reviewed. Endoscopic retrograde cholangiopancreatography (ERCP) was used to collect pancreatic juice, which was sent to the laboratory for culturing. The clinical data and bacterial culture results of the patients were then recorded and analyzed. According to the results of the pancreatic juice culture, the patients were divided into a positive bacterial culture group (n=64) and a negative bacterial culture group (n=92). It was compared the data results of two groups [age, gender, etiology, acute physiology and chronic health evaluation (APACHE) II score, cultured bacteria, complications, local complications, Balthazar computed tomography (CT) score, inflammatory factors, the use of antibiotics, drug sensitivity analysis results, and the patient's co-infection] and performed multivariate analysis to identify the clinically valuable indicators. Moreover, a receiver operating characteristic (ROC) curve was drawn to predict the model of positive pancreatic juice culture in AP.

Results: The patients in the positive bacterial culture group and the negative bacterial culture group had statistically significant differences in gender, age, body mass index (BMI), amylase, white blood cell count and the two groups of patients were comparable. A total of 156 patients were included in the study and pathogenic bacteria were cultured in the pancreatic juice of 64 patients (41.03%) and 94 strains of bacteria were found (Gram-positive bacteria, 38.30%; Gram-negative bacteria, 58.51%; fungi, 3.19%). A history of ERCP and early pancreatic necrosis were independent influencing factors of positive pancreatic juice culture. The incidence of complications, APACHE II, and inflammatory factor levels of patients with positive pancreatic juice bacterial culture were significantly higher than those of negative pancreatic juice bacterial culture ($P<0.05$). Multivariate regression and the ROC curve of pancreatic infection showed that positive pancreatic and Balthazar CT score >7 on admission were independent risk factors of pancreatic. The area under the ROC curve of patients with later pancreatic infection was 0.863 [95% confidence interval (CI): 0.769–0.957], specificity was 65.30%, sensitivity was 90.50%, and the Youden index was 0.603.

Conclusions: Bacterial culturing of pancreatic juice provides evidence of infection in the early stage of AP, which has certain significance for the anti-infective therapy of AP.

Keywords: Acute pancreatitis (AP); pancreatic juice bacteria; bacterial culture; correlation analysis

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Introduction

Acute pancreatitis (AP) is a common digestive system disease. About 80% of AP cases are mild and self-limiting. If severe AP (SAP) is infected, the mortality rate can be as high as 29.5–32% (1). Infection is an important factor leading to the death of patients with AP (2), and therefore, anti-infective therapy is crucial. Bacterial infection of pancreatic necrosis occurs in the early stage of the disease (3,4) but since pancreatic and extra-pancreatic infections often occur 2 weeks after the onset of pancreatitis, the targeted use of antibiotics in the early stage of the disease lacks pathogenic evidence and preventive drugs cannot reduce the mortality. Furthermore, the risk of multidrug-resistant bacteria and fungal infection is also increased (5). There are challenges to the use of antibiotics in the treatment of AP (6). AP is one of the most common acute abdominal diseases in clinical practice. In recent years, through the joint efforts of scholars at home and abroad, the mortality and complication rate of AP have decreased significantly, but the mortality rate of SAP is still high. Early clinical identification of SAP is essential to improve the prognosis of patients. Some scholars have proposed the model analysis of AP severity and bacterial culture, Clinical judgment of the severity of the disease will have more reference indicators. Evidence of infection obtained in the early stage is highly significant for the anti-infective therapy of AP. We found pathogens in the pancreatic juice of patients with early AP. The present study aims to provide a reference for the anti-infective therapy of AP by analyzing the distribution and clinical characteristics of pathogens in the pancreatic juice of patients with early AP. We present the following article in accordance with the STARD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6337/rc).

Highlight box

Key findings

- Following the surgical treatment of pancreatitis, pancreatic juice bacterial culture was carried out, and pathogenic bacteria with a high frequency were found.

What is known and what is new?

- Both gram-positive and gram-negative bacteria could cause pancreatitis;
- The results of the pancreatic juice bacterial culture combined with the results of the Balthazar CT score showed that gram-negative bacterial infection and the Balthazar CT score were two risk factors for pancreatitis infection.

What is the implication, and what should change now?

- The results of this study mean that we can choose effective antibiotics for drug intervention in the early treatment of patients with acute pancreatitis.

Methods

General information

Patients with acute biliary pancreatitis who underwent pancreatic stent implantation to prevent post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis and pancreatic juice bacterial culturing in the Department of Hepatobiliary surgery of the General Hospital of Ningxia Medical University from January 1, 2019, to June 30, 2020, were reviewed. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the ethics committee of the General Hospital of Ningxia Medical University (No. 2019-467). All patients voluntarily participated in this study and signed informed consent and ERCP surgery consent.

Patients and inclusion criteria

All acute biliary pancreatitis and complications in our hospital were diagnosed according to the Atlanta pancreatitis classification (as revised in 2012). The presence of gallstones with or without choledocholithiasis was confirmed by imaging examination. All patients underwent ERCP lithotomy and preventive pancreatic stent placement, and pancreatic juice was obtained for bacterial culturing and drug sensitivity testing. The included patients were older than 18 years old. We included patients with a first-time AP

infection and those with AP who could be followed-up for 6 months.

Exclusion criteria

Patients who were younger than 18 years old or had suspected specimen contamination were excluded. Furthermore, pancreatic juice culture specimens that were collected more than 1 week from the time of onset were excluded. Moreover, we also excluded patients with anesthesia or surgical contraindications, those with mental illnesses, patients with primary organ failure before onset, and those who could not cooperate with treatment, gave up treatment, or were transferred to another hospital.

Treatment plan

All patients were treated according to the Chinese guidelines for the diagnosis and treatment of AP: after admission, the patients were fasted, rehydrated, and given analgesia, enzyme inhibition, and other treatments. In addition to the above conservative treatment measures, patients with ERCP indications underwent ERCP examination and treatment within 72 hours after admission. Pancreatic stents were placed in high-risk groups to prevent post-ERCP pancreatitis, and pancreatic juice was collected for bacterial culturing.

Collection and culture of pancreatic juice

All endoscopic operations were completed by doctors with more than 10 years of surgical experience. The surgical procedure was as follows. Firstly, the endoscope reached the descending segment of the duodenum through the gastric cavity. Next, the surgeon looked for the great duodenal papilla on the inner side of the duodenum, rinsed the duodenum with normal saline, cut the knife before the sphincterotomy of the duodenal papilla, and then intubated the pancreatic duct through the guide wire. After successful intubation, an X-ray was used to confirm that the guide wire was placed along the direction of the pancreatic duct, and followed-up the incision knife to suck pancreatic juice. During the whole process, the lumen was filled with normal saline to avoid intestinal fluid pollution.

The interrupted pancreatic juice was extracted, placed into a sterile vacuum specimen collection tube, and then stored in an incubator. All specimens were sent to the laboratory for examination within 8 hours. The BacT/Alert3D blood culture and vitek-2compact bacterial identification and drug sensitivity instruments (Biomerie, France) were utilized for bacterial culturing of the pancreatic juice. The reagents applied were specifically for the instrument.

Monitoring indicators

The data were extracted by two researchers according to the pre-designed data extraction table. The extracted data contents mainly included the patient's age, gender, etiology, APACHE II score, cultured bacteria, systemic complications, local complications, Balthazar computed tomography (CT) score, inflammatory factors, the use of antibiotics, the drug sensitivity analysis results, and the patient's co-infection.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences version 20.0 (SPSS Inc., Chicago, IL, USA). Non-normally distributed metric variables were analyzed by the Kruskal-Wallis test and Mann-Whitney U-test. Logistic regression analysis was used to draw the subject-working curve [receiver operating characteristic (ROC) curve] to analyze the value of related factors. Values were expressed as the mean ± standard deviation (SD) unless stated otherwise. The measurement data of normal distribution are expressed by mean ± SD with sample *t*-test, and the measurement data of non-normal distribution were expressed by median [interquartile range (IQR)]. P value ≤ 0.05 was considered statistically significant.

Results

Basic characteristics

A total of 186 patients with pancreatic juice bacterial cultures were retrieved, of which 156 patients met the inclusion criteria, including 83 males and 73 females. The average age of the included patients was 50±17 years old, and the median time from admission to onset was 48 h (24–96 h) (*Table 1*).

Results of pancreatic juice culture and bacterial distribution

All 156 pancreatic juice samples were sent for examination,

Table 1 Basic characteristics of the included patients

Characteristic	Value
Age (years), mean ± SD	50±17
Male, n (%)	83 (53.21)
Time from admission to onset (h), median [IQR]	48 [24–96]
Alcoholism, n (%)	15 (9.62)
Diabetes, n (%)	29 (18.59)
History of ERCP, n (%)	37 (23.72)

SD, standard deviation; IQR, interquartile range; ERCP, endoscopic retrograde cholangiopancreatography.

Table 2 Distribution and composition ratio of pathogenic bacteria in pancreatic juice

Pathogen species	Number of bacteria	Constituent ratio (%)
Gram-negative bacteria	55	58.51
Escherichia coli	23	24.47
Klebsiella pneumoniae	12	12.77
Acinetobacter baumannii	5	5.32
Enterobacter	10	10.64
Pseudomonas	2	2.13
Others	3	3.19
Gram-positive bacteria	36	38.30
Enterococcus	28	29.79
Staphylococcus	3	3.19
Streptococcus	5	5.32
Fungus	3	3.19
Candida albicans	2	2.13
Candida tropicalis	1	1.06
Total	94	100.00

of which 64 (41.03%) were cultured with pathogens. A total of 94 strains were isolated from 64 positive samples, including 55 (58.51%) strains of Gram-negative bacteria, 36 (38.30%) strains of Gram-positive bacteria, and three (3.19%) strains of fungus (*Table 2*).

The pathogen susceptibility analysis results showed that all of the Gram-negative bacteria had the highest sensitivity to amikacin, among which *Escherichia coli* and *Citrobacter freundii* also had relatively high sensitivity to imipenem, cefotetan, piperacillin/tazobactam, *Klebsiella pneumoniae*, *Enterobacter cloacae*, imipenem, and cefepime. Moreover, *Acinetobacter baumannii* and *Morganella morganii* were generally resistant, and *Morganella morganii* only had a high sensitivity rate to amikacin (*Table 3*).

The drug sensitivity results of Gram-positive bacteria demonstrated that *Enterococcus faecium* had the highest sensitivity to tigecycline, vancomycin, and quinupristin/dalfopristin. *Enterococcus faecalis* had the highest sensitivity to linezolid, tigecycline, and vancomycin. Except for erythromycin and vancomycin, *Enterococcus aureus* generally exhibited high drug sensitivity to other antibiotics. *Streptococcus* has the highest drug sensitivity to linezolid and vancomycin (*Table 4*).

The drug sensitivity results of the main fungi showed that *Candida albicans* and *Candida tropicalis* had high sensitivity to fluconazole, 5-fluorocytosine, itraconazole, and voriconazole (*Table 5*).

We then compared the clinical data between the two groups with positive and negative culture results. Patients with positive and negative culture results were divided into a positive culture group and a negative culture group accordingly, and the clinical data of the two groups were compared. The results indicated that there was no significant difference in age distribution, sex ratio, body mass index (BMI) value, and time from onset to admission between the two groups. However, the C-reactive protein (CRP), D-dimer, the ratio of neutrophils to lymphocytes, APACHE II score, and Balthazar CT score in the positive culture group were higher than those in the negative culture group ($P<0.05$). The incidence of complications and duration of fever were also markedly higher in the positive culture group ($P<0.05$) (*Table 6*).

Next, we compared the clinical data between patients with and without pancreatic infection in the later stage. The patients were divided into a pancreatic infection group and a non-pancreatic infection group according to whether a pancreatic infection occurred. The clinical data of the two groups were compared and the results showed that the age distribution, sex ratio, BMI, time from onset to admission, CRP, neutrophils/lymphocytes, and APACHE II score were not significantly different between the two groups. However, the Balthazar CT score, D-dimer, fever duration, hospital stay, and fasting time in the pancreatic infection group were higher than those in the non-pancreatic infection

Table 3 Sensitivity of the main gram-negative bacteria to antibiotics

Pathogen	Antibiotics and sensitivity rate
Escherichia coli (n=23)	Amikacin (23, 100%), imipenem (22, 96%), cefotetan (21, 91%), piperacillin/tazobactam (21, 91%), tobramycin (20, 87%), nitrofurantoin (19, 83%), gentamicin (19, 83%), compound sulfamethoxazole (13, 57%), cefepime (11, 48%), ticarcillin (10, 43%), aztreonam (10, 43%), ceftriaxone (9, 39%), ceftazidime (8, 35%), ciprofloxacin (6, 26%), levofloxacin (6, 26%), cefuroxime (5, 22%), ampicillin/sulbactam (5, 22%), piperacillin (4, 17%), ampicillin (2, 9%), cefazolin sodium (1, 4%)
Klebsiella pneumoniae (n=10)	Amikacin (10, 100%), imipenem (9, 90%), cefotetan (8, 80%), cefepime (8, 80%), gentamicin (8, 80%), ciprofloxacin (7, 70%), levofloxacin (7, 70%), tobramycin (7, 70%), aztreonam (6, 60%), ceftriaxone (6, 60%), piperacillin (6, 60%), cefuroxime (6, 60%), compound sulfamethoxazole (6, 60%), ceftazidime (6, 60%), ticarcillin (6, 60%), ampicillin/sulbactam (5, 50%), cefazolin (4, 40%), nitrofurantoin (0, 0)
Enterobacter cloacae (n=4)	Amikacin (4, 100%), cefepime (4, 100%), imipenem (4, 100%), ciprofloxacin (3, 75%), levofloxacin (3, 75%), cotrimoxazole (3, 75%), piperacillin/tazobactam (3, 75%), gentamicin (2, 50%), piperacillin (2, 50%), ceftazidime (2, 50%), aztreonam (1, 25%), ceftriaxone (1, 25%), nitrofurantoin (1, 25%), ticarcillin (1, 25%)
Acinetobacter baumannii (n=5)	Amikacin (4, 80%), gentamicin (4, 80%), tobramycin (4, 80%), cotrimoxazole (3, 60%), tigecycline (3, 60%), ampicillin/sulbactam (2, 50%), ciprofloxacin (2, 50%), cefepime (2, 50%), imipenem (2, 50%), levofloxacin (2, 50%), piperacillin (2, 50%), ticarcillin (2, 50%), ceftriaxone (1, 20%)
Citrobacter freundii (n=4)	Amikacin (4, 100%), cefepime (4, 100%), nitrofurantoin (4, 100%), imipenem (4, 100%), piperacillin/tazobactam (4, 100%), aztreonam (3, 75%), ceftazidime (3, 75%), tobramycin (3, 75%), ceftriaxone (2, 50%), ticarcillin (2, 50%), gentamicin (1, 25%), piperacillin (1, 25%), ciprofloxacin (0, 0), levofloxacin (0, 0), compound sulfamethoxazole (0, 0)
Morganella morganii (n=3)	Amikacin (3, 100%), gentamicin (2, 67%), tobramycin (2, 67%), aztreonam (1, 33%), cefotetan (1, 33%), ceftriaxone (1, 33%), cefepime (1, 33%), piperacillin (1, 33%), piperacillin/tazobactam (1, 33%), ampicillin/sulbactam (0, 0), ciprofloxacin (0, 0), imipenem (0, 0), levofloxacin (0, 0), cotrimoxazole (0, 0), ticarcillin (0, 0)

Table 4 Sensitivity of the main Gram-positive bacteria to antibiotics

Pathogen	Antibiotics and sensitivity rate
Enterococcus faecium (n=12)	Tigecycline (12, 100%), vancomycin (12, 100%), quinupristin/dalfopristin (12, 100%), linezolid (11, 92%), high-level streptomycin (7, 58%), gentamicin (6, 50%), tetracycline (5, 42%), penicillin (3, 25%), ampicillin (2, 17%), nitrofurantoin (2, 17%), levofloxacin (2, 17%), ciprofloxacin (1, 8%), erythromycin (0, 0)
Enterococcus faecalis (n=5)	Linezolid (5, 100%), tigecycline (5, 100%), vancomycin (5100%), nitrofurantoin (4, 80%), gentamicin 120 (4, 80%), penicillin (4, 80%), ampicillin (3, 60%), ciprofloxacin (3, 60%), high-level streptomycin (3, 60%), levofloxacin (3, 60%), tetracycline (3, 60%), erythromycin (0, 0)
Enterococcus casseliflavus (n=2)	Ampicillin (2, 100%), ciprofloxacin (2, 100%), nitrofurantoin (2, 100%), gentamicin 120 (2, 100%), high-level streptomycin (2, 100%), linezolid (2, 100%), levofloxacin (2, 100%), penicillin (2, 100%), tetracycline (2, 100%), tigecycline (2, 100%), erythromycin (0, 0), vancomycin (0, 0)
Streptococcus (n=3)	Linezolid (3, 100%), vancomycin (3, 100%), penicillin (2, 67%), levofloxacin (2, 67%), ceftriaxone (2, 67%), ampicillin (2, 67%), erythromycin (0, 0)

Table 5 Sensitivity of the main fungi to antibiotics

Pathogen	Antibiotics and sensitivity rate
Candida albicans (n=2)	Fluconazole (2, 100%), 5-fluorocytosine (2, 100%), itraconazole (2, 100%), voriconazole (2, 100%)
Candida tropicalis (n=1)	Fluconazole (1, 100%), 5-fluorocytosine (1, 100%), itraconazole (1, 100%), voriconazole (1, 100%)

Table 6 Comparison of the clinical data of the two groups with different bacterial culture results

Characteristic	Positive bacterial culture group (n=64)	Negative bacterial culture group (n=92)	$t/z/\chi^2$	P
Age (years), mean ± SD	52.84±17.70	48.43±16.83	1.575	0.117
Gender, n (%)			0.118	0.747
Male	33 (51.56)	50 (54.35)		
Female	31 (48.44)	42 (45.65)		
BMI (kg/m^2), mean ± SD	24.58±4.27	24.71±3.97	−0.188	0.851
Time from admission to onset (h), median [IQR]	48 [24–90]	48 [24–96]	−0.532	0.594
Amylase (U/L), median [IQR]	320 [115–1035]	372 [168–861]	−0.288	0.773
Leukocytes (×10^9/L), median [IQR]	12.42 [7.66–16.35]	11.05 [7.86–14.57]	−0.996	0.319
APACHE II score, median [IQR]	6 [4–9]	5 [2–7]	−2.918	0.004
Balthazar CT score ≥7*, n (%)	11 (17.19)	2 (2.17)	11.181	0.001
D-dimer (μg/L), median [IQR]	2.7 [1.1–5.4]	1.5 [0.7–2.6]	−2.747	0.006
CRP (mg/L), median [IQR]	122 [87–195]	89 [18–123]	−3.543	0.001
Neutrophils/lymphocytes, median [IQR]	11.92 [6.81–19.79]	8.56 [4.37–12.36]	−3.064	0.002
Complications, n (%)	38 (59.38)	24 (26.09)	14.539	0.001
Systemic reaction	29 (45.31)	21 (22.83)	8.763	0.005
Local reaction	22 (34.38)	8 (8.70)	16.945	0.001
Heating duration (days), median [IQR]	2 [1–7]	1 [0.25–2]	−3.664	0.001
Length of stay (days), median [IQR]	8 [4–16]	6 [5–8]	−1.975	0.05
Pancreatic infection, n (%)	19 (29.69)	2 (2.17)	24.526	0.001

*, a Balthazar CT score ≥7 indicated that the degree of pancreatic lesions is grade III and the severity of acute pancreatitis is severe. SD, standard deviation; BMI, body mass index; APACHE, acute physiology and chronic health evaluation; CT, computed tomography; CRP, C-reactive protein; IQR, interquartile range.

group (P<0.05). The incidence of systemic inflammatory response syndrome (SIRS), multiple organ failure (MOF), pancreatic necrosis, and positive rate of pancreatic juice culture were also considerably higher in patients with pancreatic infection (P<0.05) (*Table 7*).

The multivariate regression analysis of pancreatic infection and the ROC curve included positive pancreatic juice culture, diabetes, fasting time, hospitalization time, continuous fever time, SIRS, MOF, D-dimer, and Balthazar CT score ≥7. A multivariate logistic regression equation was constructed. The results demonstrated that the effect of positive pancreatic juice culture on pancreatic infection was statistically significant [odds ratio (OR) =8.406, 95% confidence interval (CI): 1.490–47.426]. Furthermore, a Balthazar CT score ≥7 at admission had a statistically significant effect on later pancreatic infection (OR =14.632, 95% CI: 2.240–95.579) (*Table 8*). The area under the ROC curve (AUC) of these two indicators was 0.856 (95% CI: 0.758–0.954), the specificity was 90.00%, the sensitivity was 65.30%, and the Youden index was 0.553 (*Figure 1*).

Discussion

For the vast majority of SAP patients, infection is still an unavoidable problem that seriously affects the prognosis of the disease. Among them, pancreatic necrosis infection is closely related to mortality in SAP (7). In necrotizing pancreatitis, if the necrotic pancreas is secondary to an infection, the risk of death will more than double (8). The occurrence of infection in SAP is not a small probability event. In relevant reports, the overall incidence of secondary infection in SAP is 73.0–82.1%, the incidence of pancreatic

Table 7 Comparison of the clinical data between the pancreatic infection and non-pancreatic infection groups

Characteristic	Pancreatic infection group (n=21)	Non-pancreatic infection group (n=135)	$t/z/\chi^2$	P
Age (years), mean ± SD	45.71±17.15	50.59±17.25	–1.294	0.197
Gender, n (%)			0.738	0.483
Male	13 (61.90)	70 (51.85)		
Female	8 (38.10)	65 (48.15)		
BMI (kg/m^2), mean ± SD	25.71±5.23	24.50±3.88	1.182	0.239
Time from admission to onset (h), median [IQR]	48 [36–96]	48 [24–96]	–0.532	0.594
Leukocytes (×10^9/L), median [IQR]	13.10 [8.41–18.97]	11.22 [7.80–14.68]	–1.241	0.215
Apache II score, median [IQR]	7 [4–9.5]	6 [3–8]	–1.529	0.126
Balthazar CT score ≥7, n (%)	10 (47.62)	3 (2.22)	46.307	0.001
D-dimer (μg/L), median [IQR]	3.41 [1.51–7.94]	1.63 [0.74–3.07]	–2.536	0.011
CRP (mg/L), median [IQR]	122.50 [89.02–218.75]	90.00 [37.52–145.50]	–1.092	0.055
Neutrophils/lymphocytes, median [IQR]	11.94 [6.35–17.68]	8.56 [4.37–12.36]	–0.839	0.402
Pancreatic necrosis occurred, n (%)	19 (90.48)	32 (23.70)	30.45	0.001
MOF, n (%)	10 (47.62)	24 (17.78)	9.495	0.004
SIRS, n (%)	8 (38.10)	16 (11.85)	9.615	0.005
Heating duration (days), median [IQR]	5 [2–19]	1 [0–3]	–4.537	0.001
Length of stay (days), median [IQR]	15 [8.5–22]	6 [5–8]	–4.683	0.001
Fasting time (days), median [IQR]	9 [4.5–17.5]	4 [3–6]	–3.749	0.001
Pancreatic juice culture results were positive, n (%)	19 (90.48)	45 (33.33)	24.526	0.001
Diabetes, n (%)	9 (47.37)	20 (14.81)	9.433	0.005
Hypoxemia, n (%)	10 (47.62)	61 (45.19)	1.199	0.411

SD, standard deviation; BMI, body mass index; CT, computed tomography; CRP, C-reactive protein; MOF, multiple organ failure; SIRS, systemic inflammatory response syndrome; IQR, interquartile range.

infection is 39–57%, and the incidence of extra-pancreatic infection is 43.0–79.5% (1,9,10). Some scholars believe that AP is mainly caused by damage to the pancreatic barrier, which has little to do with pancreatic bacterial infection (11). Therefore, it is particularly important to clarify the relevant factors causing pancreatic infection in SAP patients, promptly grasp the distribution of pathogens caused by infection, and administer corresponding antibiotic treatment according to the distribution of pathogens.

At present, there are relatively few studies on the pathogenic bacteria of pancreatic infection. Current research shows that pancreatic and extra-pancreatic infections often occur 2 weeks after onset. At this stage, antibiotic intervention can prevent the deterioration of the disease but it cannot prevent the occurrence of infection. Thus, determining methods of identifying pathogenic bacteria in the early stage of the disease is very important (12). The discharge of pancreatic juice and bile has a similar anatomical structure. Although bile is sterile under normal conditions, some studies have found pathogenic bacteria in the bile of patients with biliary tract infections (13). Based on this, our study boldly cultured the pancreatic juice of AP patients, seeking to clarify the distribution of pathogenic bacteria in pancreatitis and provide a reference for clinical treatment (14).

In our study, the positive rate of pancreatic juice bacterial cultures was 41.03%, and 94 strains were cultured, including 55 (58.51%) Gram-negative bacteria, 36 (38.30%)

Table 8 Multivariate regression analysis of pancreatic infection

Characteristic	β	SE	Wald	P	OR	95% CI
Pancreatic juice culture positive	2.129	0.883	5.816	0.016	8.406	1.490–47.426
SIRS	0.018	1.118	0.001	0.987	1.018	0.114–9.096
MOF	0.092	0.880	0.011	0.917	1.097	0.195–6.153
Fasting time	–0.203	0.180	1.272	0.259	0.816	0.573–1.162
Hospital stay	0.282	0.185	2.329	0.127	1.325	0.923–1.903
Continuous heating time	0.004	0.116	0.001	0.975	1.004	0.799–1.260
D-dimer	0.042	0.119	0.125	0.724	1.043	0.826–1.318
Balthazar CT score ≥7 points	2.683	0.958	7.853	0.005	14.632	2.240–95.579
Diabetes	1.282	0.841	2.323	0.128	3.605	0.693–18.974

SIRS, systemic inflammatory response syndrome; MOF, multiple organ failure; CT, computed tomography; SE, standard error; OR, odds ratio; CI, confidence interval.

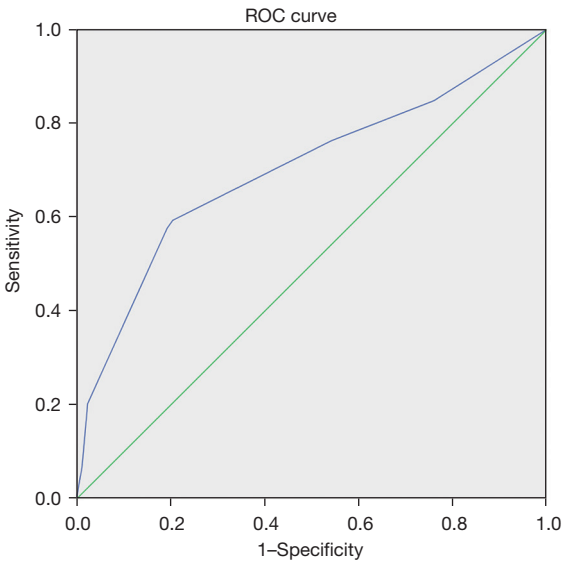

Figure 1 ROC curve of the regression model of pancreatic infection. ROC, receiver operation characteristic.

Gram-positive bacteria, and three fungi (3.19%) strains. A study has shown that regardless of whether the infection is pancreatic or extra-pancreatic, Gram-negative bacteria are the main pathogenic bacteria, and Escherichia coli is the most common (15). Also, the pathogenic bacteria found in pancreatic juice may be related to secondary infection in patients with late pancreatitis, especially pancreatic infection.

However, the proportion of fungi in our study is compared with previous studies, which is much smaller. After consulting the relevant literature for analysis, we believe that this difference is primarily due to our early pancreatic juice bacterial culture, which avoids the bacterial spectrum changes caused by the long-term preventive use of antibiotics, and the trend of this change is the transformation from Gram-negative to Gram-positive bacteria and fungus (16-19). Therefore, the proportion of fungus in pancreatic juice in the early stage of pancreatitis is low. If antibiotics are used blindly, the probability of fungal infection increases, which elevates the risk of death (5). The blind prophylactic use of antibiotics may not reduce the risk of infection and death (20); only by selecting sensitive and effective antibiotics according to the results of pathogen drug sensitivity analysis can the production of drug-resistant bacteria be reduced. In this study, pathogenic bacteria were found in pancreatic juice in the early stage of AP. Although a positive bacterial culture does not necessarily lead to infection, the results can still provide a theoretical basis for empirical antibiotic selection (21).

The pathogens of SAP secondary infection exhibit the characteristics of multi-drug resistance (22), and the selection of antibiotics is more difficult. The drug sensitivity test results in this study indicated that common gram-negative bacteria had a high sensitivity to amikacin and imipenem, but *Acinetobacter baumannii* and *Morganella morganii* were generally resistant, among which *Morganella morganii* only had high sensitivity to amikacin. Common gram-positive bacteria showed a high drug sensitivity to vancomycin, linezolid, and tigecycline, which can provide a reference basis for the selection of clinical antibiotics (23,24).

Therefore, amikacin and imipenem can be selected for gram-negative bacterial infection in SAP, and vancomycin and linezolid can be selected for suspected Gram-positive bacterial infection (25-27).

By comparing the clinical data of the two groups of patients with positive and negative cultures, we found that there were no significant differences in age distribution, sex ratio, BMI, and the time from onset to admission between the two groups. However, the indicators reflecting the severity of the disease (APACHE II score, Balthazar CT score, complication rate, etc.) were higher in positive patients than in negative patients ($P<0.05$). Some indicators that can reflect the level of inflammation and predict later pancreatic infection (e.g., CRP, D-dimer, neutrophils/lymphocytes), were also significantly higher in patients with positive cultures compared to those in negative patients ($P<0.05$). Therefore, the condition of positive patients is more serious, and the risk of pancreatic infection in the later stage is also higher.

The pathogenic bacteria in the early pancreatic juice were related to the condition and later complications of patients. On the other hand, it also showed that we cultivated pathogenic bacteria rather than polluting bacteria. There was no significant difference in leukocyte values between the two groups at admission but CRP, PCT, and neutrophils/leukomonocyte were significantly different in leukocytes, and thus, leukocytes were less sensitive than CRP and the other inflammatory factors in the early evaluation of AP and the prediction of infection. Therefore, we should pay more attention to these inflammatory indicators in anti-infective therapy and pancreatic juice was sterile under normal conditions (28).

We conducted a multivariate regression analysis of pancreatic infection to explore the influence of pathogenic bacteria in pancreatic juice on pancreatic infection in patients at a later stage. The results showed that positive pancreatic juice culture and Balthazar CT score ≥ 7 were independent risk factors for pancreatic infection. The ROC curve combining the two risk factors showed that a positive pancreatic juice culture and a Balthazar CT score ≥ 7 had a certain predictive value for pancreatic infection. Imaging plays an important role in the evaluation of the AP condition. CT examination can not only accurately display the anatomical morphology of SAP lesions and evaluate the degree of pancreatic necrosis, as a non-invasive examination method, it can also dynamically monitor the changes in patients' conditions and provide guidance for clinical treatment and prognostic evaluation. The Balthazar CT score is an evaluation method established according to the degree of involvement of the peripancreatic and retroperitoneal space. Some scholars believe that this method should be the first choice to evaluate the severity of AP patients in the early stage; however, the Balthazar CT score results are affected by the subjective factors of the operator. Therefore, under the premise of allowable conditions, routine pancreatic juice bacterial culture should be carried out. Then, the occurrence of pancreatic infection in the later stage of AP can be predicted according to the culture results combined with the Balthazar CT score, which has a positive significance for the anti-infection treatment of AP.

In conclusion, we found pathogens in the pancreatic juice of 41.03% of patients with AP, among which Gram-negative bacteria were the most prevalent, followed by Gram-positive bacteria and fungi. The presence of pathogens in pancreatic juice is closely related to the patient's condition and prognosis, and the presence of pathogens in pancreatic juice is an independent risk factor for pancreatic infection in the later stage of the disease. If antibiotics can be used in the early stage of pancreatitis according to the results of pancreatic juice culture and drug sensitivity testing, this may provide better outcomes for patients with AP (29).

Conclusions

Bacterial culture of pancreatic juice can obtain evidence of infection in the early stage of AP, which has certain reference significance for anti-infection treatment of AP. Our study first reported the distribution and clinical characteristics of pancreatic juice bacteria in AP. Pancreatic juice bacterial cultures can provide evidence of infection in the early stage of AP, which provides a reference for the anti-infective therapy of AP.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://atm.amegroups.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the ethics committee of the General Hospital of Ningxia Medical University (No. 2019-467). All patients voluntarily participated in this study and signed informed consent and ERCP surgery consent.

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