Bacteremia in Patients With Acute Pancreatitis as Revealed by 16S Ribosomal RNA Gene-Based Techniques*

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Objectives: To define the characteristic of bacteremia in patients with acute pancreatitis and determine its possible association with the disease severity.

Design: A prospective controlled study.

Setting: ICU of Jinling Hospital, China.

Patients: A total of 48 patients with mild or severe acute pancreatitis were enrolled in the study.

Interventions: None.

Measurements and Main Results: Samples of peripheral blood were collected from the patients at 4 or 5 and 9 or 10 days after acute pancreatitis was definitely diagnosed. Resulting DNA from the blood was analyzed using denaturing gradient gel electrophoresis, and separated fragments were sequenced for identification of bacterial species. Bacterial DNA was detected in peripheral blood from 68.8% of patients with acute pancreatitis, and more than half (60.4%) of the patients encountered polymicrobial flora. Translocated bacteria in patients with acute pancreatitis were primarily constituted of opportunistic pathogens derived from the gut, including *Escherichia coli*, *Shigella flexneri*, *Enterobacteriaceae bacterium*, *Acinetobacter lwoffii*, *Bacillus coagulans,* and *Enterococcus faecium*. The species of circulating bacteria shifted remarkably among the patients with different severity. The presence of the bacteremia correlated positively with the Acute Physiology and Chronic Health Evaluation-II scores of patients with acute pancreatitis ($r = 0.7918$, $p < 0.0001$).

Conclusions: This study provides a detailed description on the prevalence of bacteremia and characteristic of bacterial species in patients with acute pancreatitis. We demonstrate an association between the bacteremia and the disease severity, which enables us to better understand a potential role of bacterial translocation in the pathogenesis of septic complication in acute pancreatitis. (*Crit Care Med* 2013; 41:1938–1950)

Key Words: bacteremia; bacterial translocation; denaturing gradient gel electrophoresis; DNA sequencing; severe acute pancreatitis

Infection and sepsis are the severe complications contributing to most late deaths in severe acute pancreatitis (SAP) (1, 2). The organisms causing infection are common enteric bacteria in acute pancreatitis (AP) (3, 4), which have been generally supported by animal experiments (5–7). Bacterial translocation is defined as translocation of bacteria that are normally resident within the lumen of the intestinal tract across the epithelial mucosa to extraintestinal sterile circumstances and act as a source of sepsis in different sites (7–11). The animal studies have shown that bacterial translocation occurs commonly in various situations (5–7, 11–13). Increased intestinal permeability has been described in patients with SAP (14–16). Most bacteria associated with infections in AP are enteric in origin, implying that the bacterial translocation could play a role in the pathogenesis of these infections (3–8). Despite its clinical importance, bacterial translocation has received little attention in SAP in laboratory research. Attempts to confirm the association between bacterial translocation and the risks of infections in patients with SAP remain largely unproven.

*See also p. 2048.

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Drs. Qiurong Li and Jieshou Li conceived and designed the study. Mr. Wang, Ms. Tang, Ms. He, and Dr. Ning Li performed the experiments. Dr. Qiurong Li and Mr. Wang analyzed the data and wrote and revised the manuscript. All authors approved the final version.

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Unlike animal models, the thesis of bacterial translocation in the patients has been largely unsuccessful. Because sequential culture of tissues, including mesenteric lymph nodes, are not possible in patients, and direct culture is available only in patients undergoing laparotomy. Furthermore, culture-based methods identify only 20% to 30% of bacteria in a complex bacterial community in the gut (17, 18). The systemic circulation is the site least found to harbor bacteria, and cultures of blood specimens from patients with SAP are often sterile even in the presence of infected pancreatic necrosis (19). It is possible that bacteria may translocate into the systemic circulation, albeit in small numbers, but they are not detected by conventional culture techniques (17, 18). Identification of bacterial DNA in blood samples by polymerase chain reaction (PCR) has been described in bacterial translocation (19–22). However, PCR fails to detect several microorganisms in a single specimen (22). Genetic fingerprints based on 16S ribosomal DNA (rDNA), such as denaturing gradient gel electrophoresis (DGGE), provides a culture-independent approach to determine bacterial community structure of gut microbiota (23–25). Recently, this method has been used to investigate the abundance and biodiversity of bacterial microbiota in health and disease (23–25).

TABLE 1. Clinical Characteristics and Antibiotic Use of the Patients

Variable	Total	Mild Acute Pancreatitis APACHE-II Score < 8	Severe Acute Pancreatitis	
			APACHE-II Score 8–12	APACHE-II Score > 12
Number of patients, *n*	48	11	24	13
Age, yr (mean ± SD)	49.3 ± 14.6	41.3 ± 12.7	53.3 ± 15.0	48.5 ± 13.1
Gender (male/female)	23/25	8/3	8/16	7/6
Etiology, *n* (%)				
Biliary disease	22 (45.8)	5 (45.4)	10 (41.7)	7 (53.8)
Hyperlipidemia	10 (20.9)	3 (27.3)	4 (16.7)	3 (23.1)
Alcohol	1 (2.1)	0 (0)	1 (4.2)	0 (0)
Unknown cause	15 (31.2)	3 (27.3)	9 (37.5)	3 (23.1)
Antibiotic use, *n* (%)				
Piperacillin (4.5 g, tid)	26 (54.2)	4 (36.4)	15 (62.5)	7 (53.8)
Sulbactam (4.5 g, tid)				
Ornidazole (0.5 g, bid)				
Cefotaxime (3.0 g, tid)	10 (20.8)	2 (18.2)	5 (20.8)	3 (23.1)
Sulbactam (4.5 g, tid)				
Ornidazole (0.5 g, bid)				
Meropenem (0.5g × 4/d)	5 (10.4)	1 (9.1)	2 (8.3)	2 (15.4)
Vancomycin (0.5 g, bid)				
Ornidazole (0.5 g, bid)				
Vancomycin (0.5 g, bid)	3 (6.3)	1 (9.1)	1 (4.2)	1 (7.7)
Diflucan (0.2 g × 1/d)				
Ornidazole (0.5 g, bid)				
Tienam (3.0 g, bid)	2 (4.2)	1 (9.1)	1 (4.2)	0 (0.0)
Ornidazole (0.5 g, bid)				
Metronidazole (0.2 g, bid)	1 (2.1)	1 (9.1)	0 (0.0)	0 (0.0)
No antibiotic	1 (2.1)	1 (9.1)	0 (0.0)	0 (0.0)
APACHE II score (mean ± SD)	9.6 ± 4.2	3.7 ± 1.6	9.5 ± 1.4	14.8 ± 2.0
ICU stay, d (mean ± SD)	16.3 ± 7.2	6.6 ± 6.2	17.0 ± 3.8	23.0 ± 3.2
Mortality, *n* (%)	1 (2.1)	0 (0)	1 (4.2)	0 (0)

APACHE = Acute Physiology and Chronic Health Evaluation.

In this study, using 16S rDNA-based molecular techniques, we aimed to investigate the characteristic of the bacteremia and to extend our understanding for its role played in the pathogenesis of AP. We described for the first time that the bacteremia was closely related to disease severity, identifying it as an important determinant for systemic infections in AP. Our findings might facilitate future efforts to uncover the origin of sepsis and to search for a therapeutic strategy of septic complication.

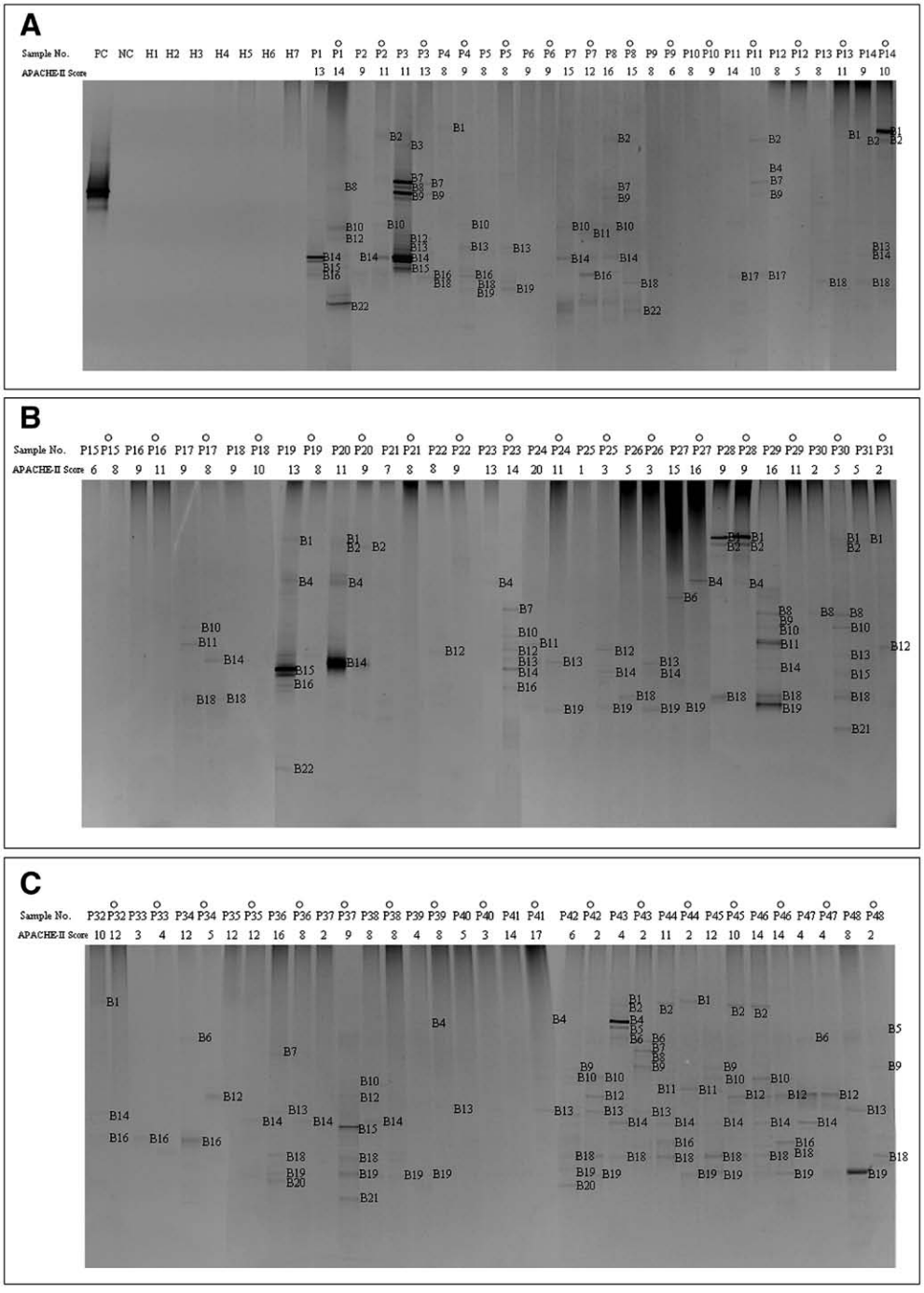

Figure 1. Molecular detection of the bacterial DNA in peripheral blood specimens from the patients with acute pancreatitis (AP) ($n = 48$) and healthy subjects ($n = 7$). Representative denaturing gradient gel electrophoresis (DGGE) profiles of the blood samples from healthy controls and patients with AP (**A**, **B**, **C**). DNA of whole blood was amplified by polymerase chain reaction with bacterial universal primers, and the amplicons were analyzed using DGGE with a denaturant gradient from 30% to 50%. PC = positive control; NC = negative control, HC1–HC7 = the samples from seven healthy controls, APACHE = Acute Physiology and Chronic Health Evaluation. The letter "P" represents the blood sample collected from patients with AP, and the figures imply the serial numbers of the patients. The sign "O" indicates the sample collected at 9 or 10 days and another at 4 or 5 days.

MATERIALS AND METHODS

Patients

This prospective observational study included 48 patients with AP, who were admitted to Jinling Hospital between January 2011 and September 2012. Patients were recruited if the onset of upper abdominal pain was within 48 hours at admission. The diagnosis of AP was estimated on the first 12 hours of admission by presence of acute upper abdominal pain, serum amylase, and/or lipase greater than three times the upper limit of normal and contrast-enhanced CT (26). SAP was defined according to the Atlanta clinical criteria (26). The severity of the disease was evaluated based on the criteria of Acute Physiology and Chronic Health Evaluation (APACHE)-II (14, 27). A detailed description for the characteristics of the patients was summarized in **Table 1**. The patients were treated on the basis of standardized protocols of interdisciplinary management, including gastrointestinal decompression, IV fluids, nutritional support, and/or organ system support. The patients received antibiotic prophylactic treatment within 48 hours after AP onset and continued until unequivocal clinical improvement (Table 1). Seven healthy volunteers (three men and four women, 46.6 ± 16.3 yr) acted as controls. The subjects were in good health and had no history of either pancreatic or gastrointestinal disease. This study was approved by the Human Subjects Institutional Committee of Jinling Hospital. Written informed consent was obtained from the participants.

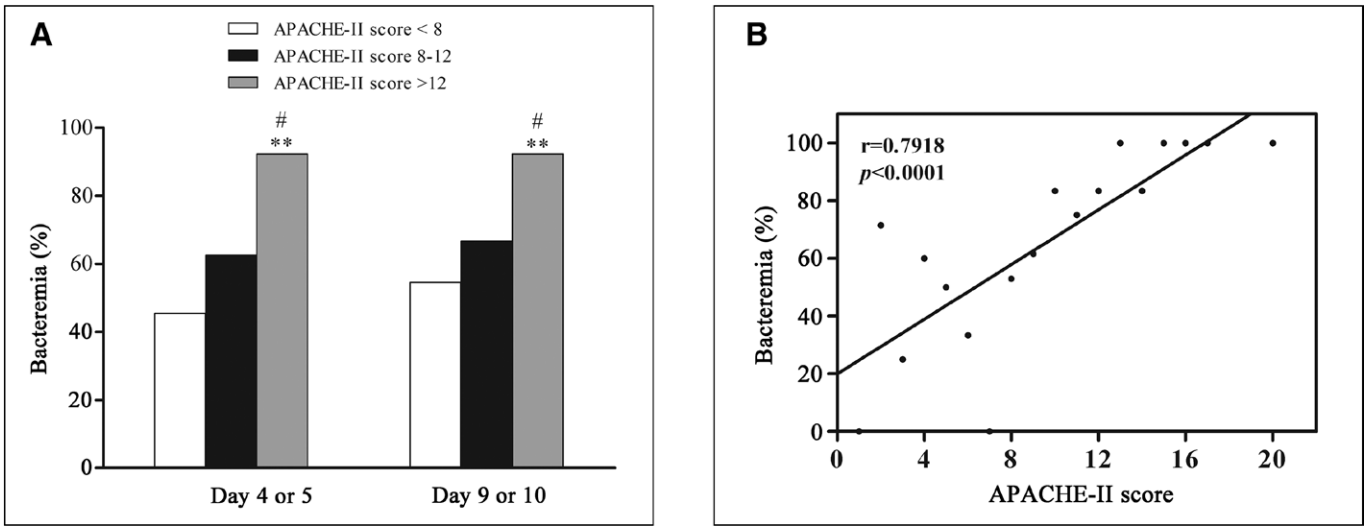

Figure 2. Relationship between the prevalence of bacteremia and the severity of acute pancreatitis (AP). **A**, Changes in the prevalences of the bacteremia in patients with AP with various Acute Physiology and Chronic Health Evaluation (APACHE)-II scores. $^{**}p < 0.01$, compared with the patients with mild AP (APACHE-II score < 8); $\#p < 0.05$, compared with the patients with APACHE-II score 8–12 points. **B**, The rate of the bacteremia correlated positively with APACHE-II scores of patients with AP. *White bar* = APACHE-II score < 8, *black bar* = APACHE-II score 8–12, *gray bar* = APACHE-II score > 12, *Dots* represent the prevalence of bacteremia in the patients with different APACHE-II scores.

TABLE 2. The Sequence Analysis of Denaturing Gradient Gel Electrophoresis Bands From Blood Samples

Band	Closest Relative	Strain	GenBank Accession	Identity (%)
B1	*Staphylococcus epidermidis*	Fussel	NR_036904	99
B2	*Staphylococcus aureus*	MVF-7	NR_036828	100
B3	*Staphylococcus cohnii*	CK27	NR_037046	100
B4	*Actinobacillus succinogenes*	130Z	NC_009655.1	100
B5	*Enterobacter cloacae* subspecies dissolvens	SDM	NC_018079.1	100
B6	*Eubacterium rectale*	ATCC 33656	NC_012781.1	99
B7	*Klebsiella pneumoniae*	DSM 30104	NR_036794	100
B8	*Serratia marcescens*	ATCC 13880	NR_041980	99
B9	*Pseudomonas aeruginosa*	NAPCC-1	FJ226424.1	100
B10	*Acinetobacter lwoffii*	WJ10621	AFQY01000001	99
B11	*Enterococcus faecium*	TX0133a01	NZ_GL476170	99
B12	*Bacillus coagulans*	36D1 ctg473	NZ_AAWV02000001	99
B13	*Shigella flexneri*	J1713	AFOW01000002	100
B14	*Escherichia coli*	PCN033	AFAT01000118	100
B15	*Streptococcus pneumoniae*	670-6B	NC_014498.1	99
B16	*Enterobacteriaceae bacterium*	9_2_54FAA	NZ_ADCU01000033	100
B17	*Stenotrophomonas maltophilia*	IAM 12423	NR_041577	99
B18	*Pseudomonas putida*	ND6	NC_017986.1	99
B19	*Neisseria meningitidis*	NZ-05/33	NC_017518.1	99
B20	*Methylobacterium radiotolerans*	JCM 2831	NC_010505.1	98
B21	*Burkholderia* species	YI23	NC_016589.1	99
B22	*Enterobacter aerogenes*	JCM1235	NR_024643	99

TABLE 3. Distributions of the Bacteria Identified by DNA Sequencing

Phylum	Class	Order	Family	Genus
Proteobacteria	γ-Proteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia
				Shigella
				Enterobacter
				Erwinia
				Klebsiella
				Serratia
		Pasteurellales	Pasteurellaceae	Actinobacillus
		Pseudomonadales	Pseudomonadaceae	Pseudomonas
			Moraxellaceae	Acinetobacter
		Xanthomonadales	Xanthomonadaceae	Stenotrophomonas
	α-Proteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium
	β-Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia
		Neisseriales	Neisseriaceae	Neisseria
Subtotal				
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus
				Staphylococcus
		Lactobacillales	Enterococcaceae	Enterococcus
			Streptococcaceae	Streptococcus
	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium
Subtotal				
Total				

Sample Collection and Blood Culture

Peripheral venous blood samples were obtained from the patients on day 4 or 5 and day 9 or 10 after AP was definitely diagnosed. Two 10-mL aliquots of venous blood were collected for standard bacterial culture. Microorganisms were identified according to clinical laboratory methods. A further 2 mL of blood was used for detection of bacterial DNA.

DNA Extraction and PCR Amplification

Extraction of whole-blood DNA was conducted using the QIAamp Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instruction. The yield and purity of DNA were measured by reading A260 and A280 in a Beckman spectrophotometer (Beckman-Coulter, Fullerton, CA). Isolated DNA was used as a template to amplify the hypervariable V6–V8 regions of 16S ribosomal RNA (rRNA) gene. Universal primers were F968-GC (5′-GC-clamp-AACGCGAAGAACCTTAC-3′) and R1401 (5′-GGTGT-GTACAAGACCC-3′) (24). To avoid false-positive results, positive and negative controls were performed in duplicate in each assay. DNA from *Klebsiella pneumoniae* (an isolate from a positive blood culture) was added as positive control and sterile water and PCR mixture (without template) as a negative control (21, 28). An aliquot of DNA (100 ng) was added into a reaction mixture, and PCR reactions were carried out with a touchdown thermocycling program (24). The purity and correct size of the resulting PCR amplicons (approximately 500 bp) were assessed on 1% agarose gels, stained with ethidium bromide (5 μg/mL), and visualized under ultraviolet (UV) light.

Species	Mild Acute Pancreatitis	Severe Acute Pancreatitis	
	< 8 (*n* = 11)	8–12 (*n* = 24)	> 12 (*n* = 13)
Escherichia coli	5	11	11
Shigella flexneri	5	4	8
Enterobacter aerogenes	0	0	2
Enterobacter cloacae subspecies dissolvens	2	0	0
Enterobacteriaceae bacterium	2	5	6
Klebsiella pneumoniae	2	4	3
Serratia marcescens	0	2	2
Actinobacillus succinogenes	1	0	1
Pseudomonas aeruginosa	6	2	2
Pseudomonas putida	11	5	4
Acinetobacter lwoffii	6	5	5
Stenotrophomonas maltophilia	0	0	3
Methylobacterium radiotolerans	2	0	0
Burkholderia species	1	1	2
Neisseria meningitidis	7	7	3
	50	47	51
Bacillus coagulans	5	4	6
Staphylococcus aureus	1	9	2
Staphylococcus cohnii	0	1	4
Staphylococcus epidermidis	1	4	1
Enterococcus faecium	2	3	5
Streptococcus pneumoniae	2	0	1
Eubacterium rectale	2	1	1
	13	22	20
	63	69	71

Denaturing Gradient Gel Electrophoresis

DGGE analysis of PCR amplicons was performed on a D-Code universal mutation detection system (Bio-Rad, Hercules, CA). The fragments of 16S rRNA gene were separated with 8% polyacrylamide gels (acrylamide:bisacrylamide, 37.5:1) containing a linear urea-formamide gradient from 30% to 50%. Electrophoresis was conducted in 1 × Tris acetate EDTA buffer (0.8 mM Tris, 0.4 mM acetic acid, 0.8 mM EDTA) at 60°C at a constant voltage of 120 V for 7.5 hours. DNA bands were stained by SYBR Green I (Invitrogen, Eugene, OR) and photographed by UV transillumination with ChemiDOC XRS instrument (Bio-Rad).

Analysis of DGGE Profile

Digitalization of the profiles was done by QuantityOne 4.6 software package (Bio-Rad). After the background was subtracted and lanes were normalized, individual bands were marked automatically by the software. Bands occupying the same position in different lanes were matched and identified as the same band type, using the match tolerance of 0.5% (29). The quantitative information derived from the presence/absence (1/0) of the band per band type per sample was exported as a data matrix. The total numbers of DGGE bands in each lane were then documented. To evaluate possible variances of bacterial composition in peripheral blood

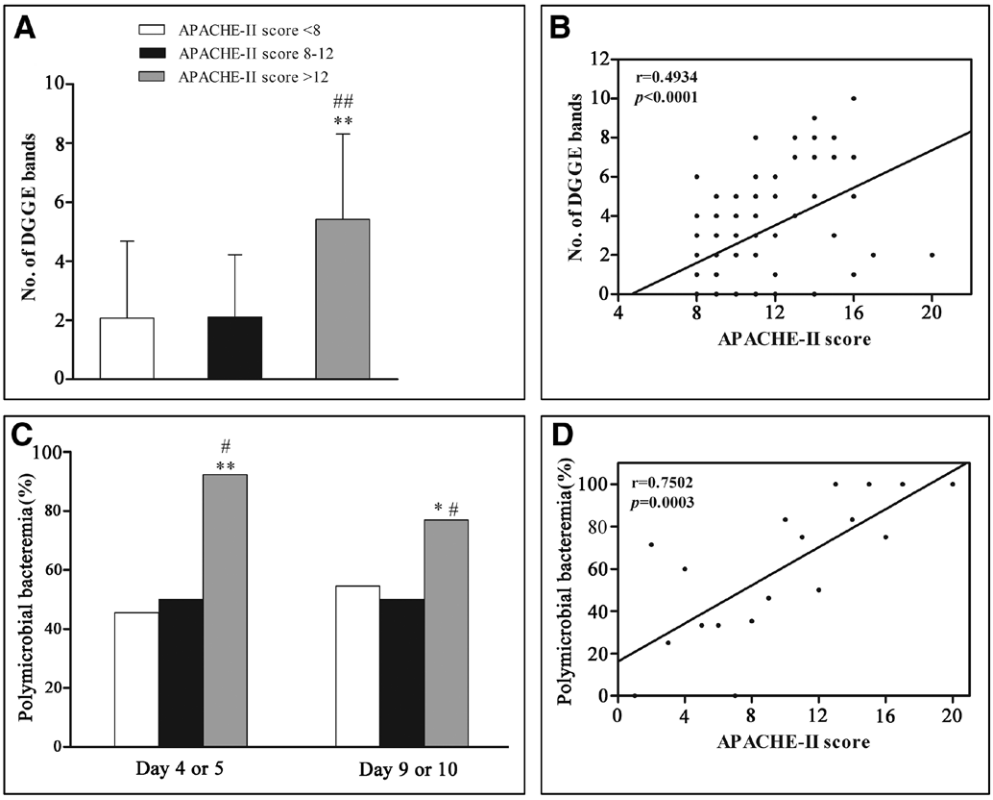

Figure 3. The presence of polymicrobial bacteremia in patients with acute pancreatitis (AP) and its association with the severity of the disease. **A**, Alterations in the numbers of denaturing gradient gel electrophoresis (DGGE) bands in the patients with different Acute Physiology and Chronic Health Evaluation (APACHE)-II scores. **B**, Positive correlation between the numbers of DGGE bands and APACHE-II scores of the patients. *Dots* represent the number of DGGE bands in the patients with severe acute pancreatitis. **C**, Prevalences of polymicrobial flora among the patients with different severity. **D**, The prevalence of polymicrobial bacteremia in peripheral blood is associated with the disease severity. *Dots* represent the prevalence of polymicrobial bacteremia in the patients with different APACHE-II scores. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the patients with mild AP (APACHE-II score < 8); $\#p < 0.05$, $\#\#p < 0.01$, compared with the patients with APACHE-II score 8–12 points. *White bar* = APACHE-II score < 8, *black bar* = APACHE-II score 8–12, *gray bar* = APACHE-II score > 12.

among the patients, a principal component analysis (PCA) was conducted using CANOCO 4.5 software package (Microcomputer Power, Ithaca, NY) based on the data matrix mentioned above.

Determination of DNA Sequence and Phylogenetic Analysis

To determine bacterial species corresponding to DGGE bands, DNA fragments were excised and reamplified using the thermal conditions described above. The reamplified fragments were subcloned onto a pGEM-T vector (Promega, Madison, WI) and sequenced as previously described (25, 30). The retrieved sequences were compared in the GenBank database using a Basic Local Alignment Search Tool algorithm to search for the closest bacterial relatives. Multiple sequence alignments of the nucleotide sequences were performed using a CLUSTAL W program (http://bips.u-strasbg.fr/fr/Documentation/ClustalW/clustalw.html) (31). A neighbor-joining tree was generated on the basis of base-pair sequences using MEGA software (version 4.0) (http://www.megasoftware.net) (32).

Statistical Analysis

Quantitative data were reported as mean ± SD. The prevalence of bacteremia was compiled and expressed as percentage. Statistical differences of basal characteristics between groups were calculated using one-way analysis of variance and Dunnett *t* test for continuous variables and the chi-square test for categorical data. The association of bacterial presence with the severity of the disease was determined by linear regression analysis using Pearson test. A *p* value of less than 0.05 was considered significant. All statistical analyses were performed using the SPSS 16.0 software (SPSS, Chicago, IL).

RESULTS

Molecular Detection of Bacterial DNA in Peripheral Blood

The presence of bacterial DNA in patients with mild and severe AP was evaluated using 16S rDNA-based technique. As shown in the DGGE profiles, there was no band in lanes 3–9, indicating negative amplification in healthy controls and validating the specificity of the molecular technique for detection of bacterial DNA in blood samples (**Fig. 1*A***). A total of 66 (68.8%) blood specimens consisted of at least one band, and the bacteremia was confirmed in these patients (Fig. 1). In the patients with an APACHE-II score greater than 12 points, bacteria were detected in 12 patients (92.3%) at 4 or 5 days and 9 or 10 days post diagnosis of AP (Figs. 1 and **2*A***). For the patients with an APACHE-II score 8–12 points, microbial DNA was identified in 62.5% and 66.7% of these patients at both time points, respectively. Only five of the patients (45.5%) with mild AP were documented positive at 4 or 5 days and six patients (54.5%) at 9 or 10 days, which were significantly decreased versus those with greater than 12 points ($p < 0.01$). In addition, there was no significant difference in the prevalence of the bacteremia in these patients between 4 or 5 days and 9 or 10 days. The prevalence of the bacteremia was positively correlated with APACHE-II scores of patients with AP ($r = 0.7918$, $p < 0.0001$) (**Fig. 2*B***), suggesting a potential association between the bacteremia and the severity of the disease.

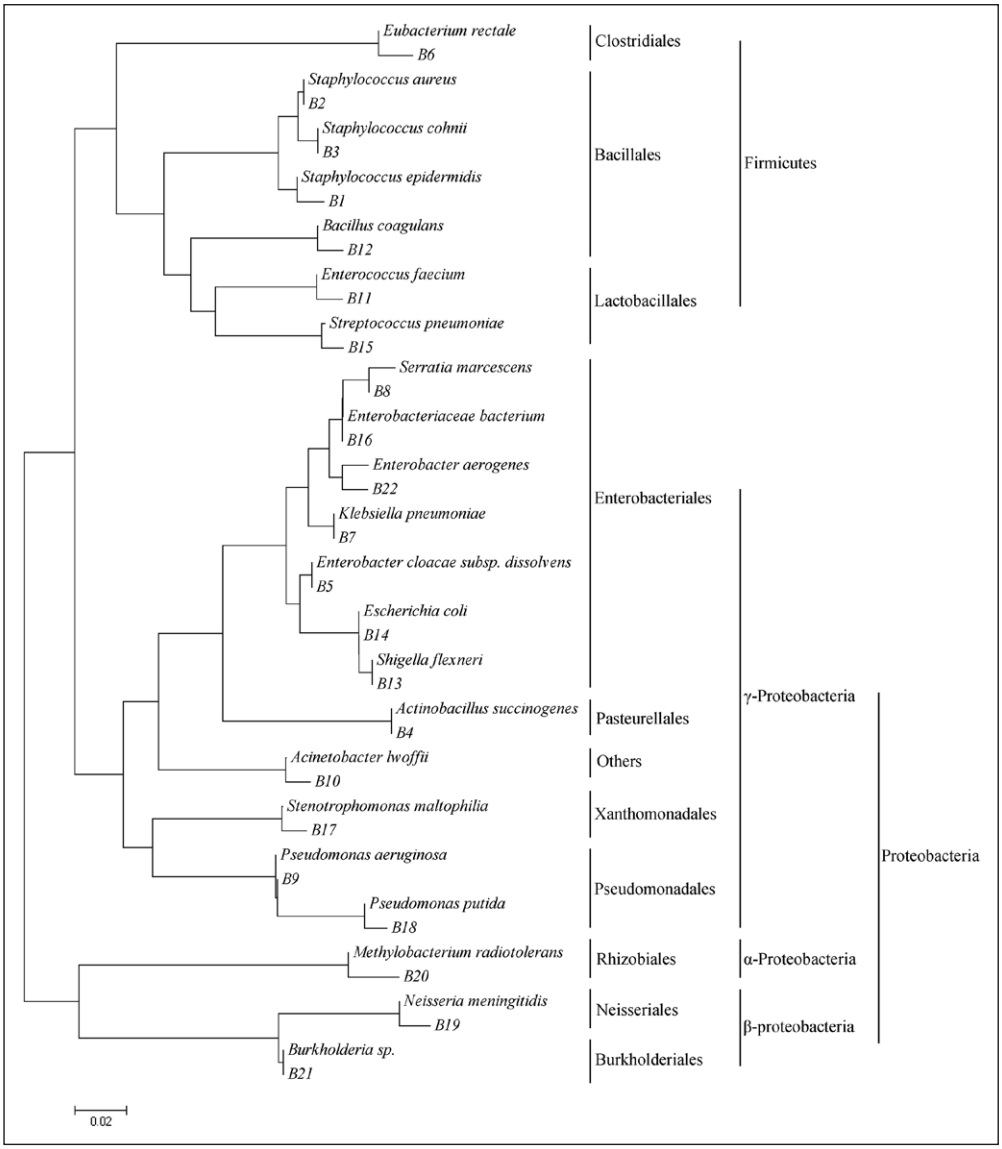

Figure 4. Phylogenetic analysis of the partial 16S ribosomal DNA sequences obtained from the separated DNA fragments in the patients with acute pancreatitis. The sequences are aligned with those of reference strains retrieved from the GenBank database using the Basic Local Alignment Search Tool. These bacterial species were divided into two clusters: the phylum Proteobacteria and Firmicutes. The scale bar represents the genetic distance.

Assessment of Polymicrobial Bacteremia by DGGE Technique

The polymicrobial bacteremia was indicated by the presence of multiple DNA bands in the molecular profiles. A remarkable increase in the total numbers of the fragments was observed in the patients with an APACHE-II score greater than 12 points versus the patients with 8–12 points (5.42 ± 2.89 vs 2.10 ± 2.12, $p < 0.001$) (**Fig. 3*A***). The band numbers were 2.07 ± 2.61 in the mild patients, and less than in those with a score greater than 12 points ($p < 0.001$). Furthermore, the numbers of the DNA fragments were related positively with APACHE-II scores of the patients with SAP ($r = 0.4934$, $p < 0.0001$) (**Fig. 3*B***).

The prevalence of polymicrobial bacteremia was expressed as the percentage of the positive samples with multiple bands. There were multiple fragments being found in 58 (60.4%) of the specimens (Fig. 1). Twelve of the patients (92.3%) with greater than 12 points had multiple bands at 4 or 5 days and 10 patients (76.9%) at 9 or 10 days, whereas half of the patients (50.0%) with 8–12 points were identified as having polymicrobial bacteremia (**Fig. 3*C***). In the patients with mild AP, polymicrobial flora was observed in 45.5% at 4 or 5 days and in 54.5% at 9 or 10 days, which was lower than in those with greater than 12 points ($p < 0.01$). Interestingly, the prevalence of polymicrobial bacteremia was positively associated with the APACHE-II scores in patients with AP ($r = 0.7502$, $p = 0.0003$) (**Fig. 3*D***). Collectively, polymicrobial bacteremia in systemic circulation was probably linked to the disease severity in AP.

Bacterial Species Translocated into Systemic Circulation

To further define the characteristic of bacterial species translocated in systemic circulation, specific DNA fragments were sequenced. The closest bacterial relatives corresponding to the fragments were divided into two clusters: 15 species in the Proteobacteria phylum and seven species in the Firmicutes phylum (**Fig. 4** and **Table 2**). Of the 203 clones identified, 148 sequences (72.9%) were included in the Proteobacteria phylum and the remaining 55 sequences (27.1%) in the Firmicutes phylum, with the Enterobacteriales order (36.5%) predominating and the Pseudomonadales order (22.7%) and the Bacillales order (18.7%) as the next most abundant (**Table 3**). We then determined the feature of bacteria presented in systemic circulation at species level. As shown in Table 3, *Escherichia coli* (B14), a member of the Enterobacteriales order, was detected in 27 specimens and was the most common organism in peripheral blood. Other organisms of the order, such as *Shigella flexneri* (B13), *Enterobacteriaceae bacterium* (B16), and *K. pneumoniae* (B7), were found in more than 10% of patients. In the Pseudomonadales order,

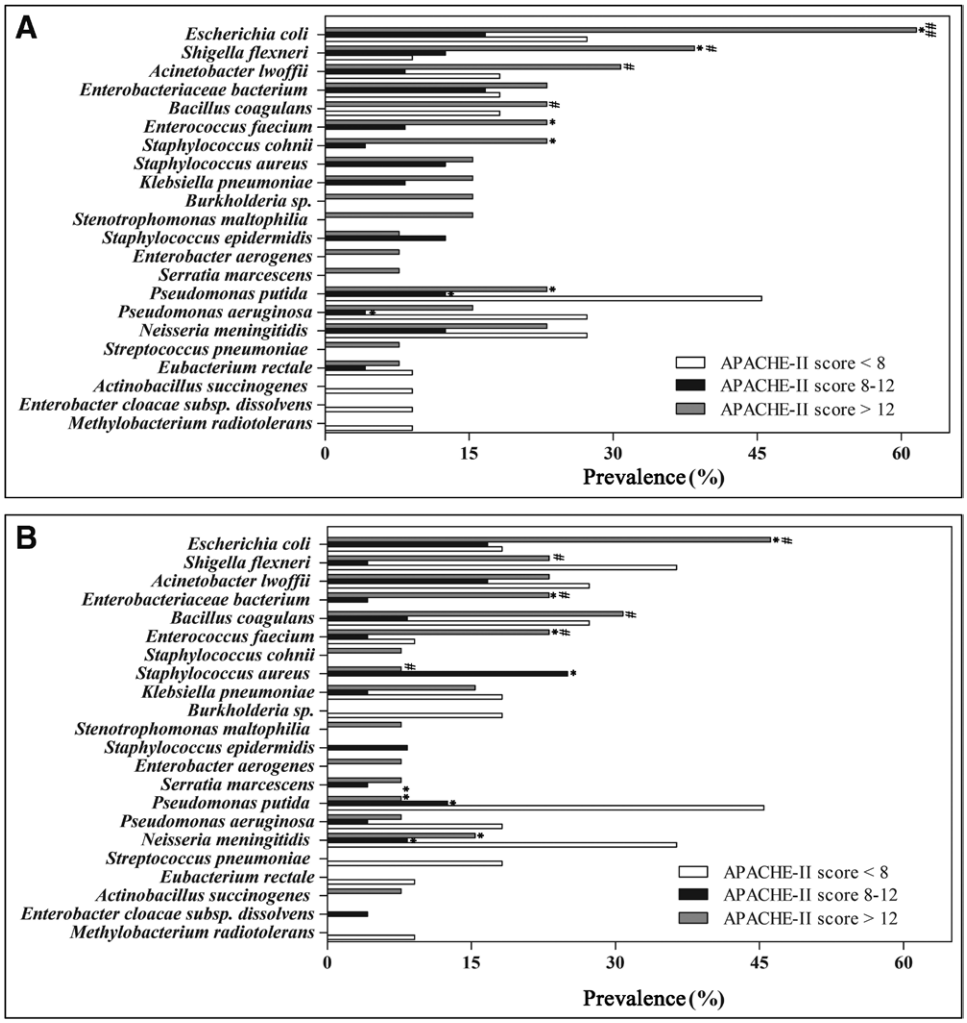

Figure 5. The prevalence of bacteria presented in patients with acute pancreatitis (AP) with different Acute Physiology and Chronic Health Evaluation (APACHE)-II scores. Separated deoxyribonucleic acid fragments are sequenced, and the retrieved sequences are compared in the GenBank database to identify the closest bacterial relatives. The histogram showing the frequency of bacteria in AP patients with different severity at 4 or 5 days (**A**) and 9 or 10 days (**B**). *$p < 0.05$, compared with the patients with mild AP (APACHE-II score < 8). *White bar* = APACHE-II score < 8, *black bar* = APACHE-II score 8–12, *gray bar* = APACHE-II score > 12.

the predominant clones identified were *Pseudomonas putida* (B18), *Pseudomonas aeruginosa* (B9), and *Acinetobacter lwoffii* (B10). Gram-positive organisms, *Bacillus coagulans* (B12), *Enterococcus faecium* (B11), and *Staphylococcus aureus* (B2) were also often observed in the patients. Most of these organisms were commonly resident in the enteric tract, indicating that bacterial translocation might be a major source of bacteremia in patients with AP.

Specific Distribution of Translocated Bacteria

We further investigated whether the types of circulating bacteria changed with disease manifestation. In the patients with an APACHE-II score greater than 12 points, *E. coli* was detected in 61.5% and 46.0% of the patients at 4 or 5 days and 9 or 10 days, respectively; therefore, it was probably the most important pathogen responsible for the systemic infection (**Fig. 5**). In addition, enteric commensal bacteria, including *S. flexneri*, *E. bacterium*, *A. lwoffii*, *B. coagulans,* and *E. faecium*, were also involved in more than 23.1% of these patients. However, *P. putida*, *Neisseria meningitidis,* and *P. aeruginosa* appeared to be more prevalent at 4 or 5 days. Although these bacteria mentioned above were shared among patients with AP, a clear reduction in their prevalences other than *Pseudomonas* species was observed in the patients with 8–12 points (Fig. 5). Similarly, *E. coli*, *E. bacterium,* and *E. faecium* were less present in systemic circulation in patients with mild AP. These results demonstrated that the specific distribution of bacteria was likely associated with the severity of pancreatitis.

Variances of Bacterial Composition in the Patients

The difference of microbial composition presented in systematic circulation between patients with mild and severe AP was assessed by PCA. According to the ordination diagram, the first two principal components (PC1 and PC2) explained 34% of inter-sample variability in bacterial composition (**Fig. 6**). Most of the samples from the patients with greater than 12 points were clustered in an individual dataset. The specimens from the patients with 8–12 points were distributed into a separated cluster and the patients with mild AP in another cluster. The data indicated alterations in the bacterial composition among the patients with various severities.

Comparative Analysis of Bacteremia by Cultivation and Molecular Techniques

Twenty-one blood samples were documented as positive by culture method, which was far less than that from the DGGE analysis (21.9% vs 68.8%, $p < 0.01$) (**Fig. 7*A***). Six of the positive blood cultures (23.1%) were assigned in the patients with greater than 12 points, 11 in the patients with 8–12 points (22.9%), and four in the patients with mild AP (18.2%), whereas DGGE detection showed prevalences of 92.3%, 64.6%, and 50.0% in these patients, respectively ($p < 0.05$). Only three of the patients with greater than 12 points (11.5%) were determined to have polymicrobial bacteremia by cultivation methods and two in the cases with 8–12 points (4.8%), which were significantly lowered compared with the DGGE analysis ($p < 0.05$) (**Fig. 7*B***). In addition, we showed

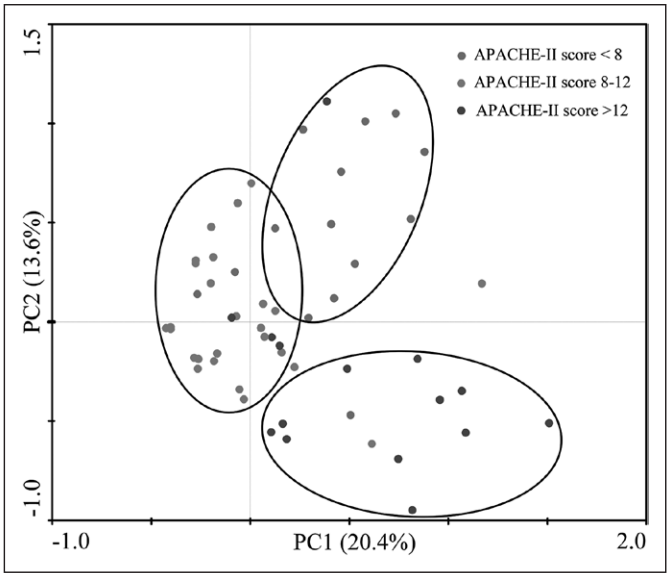

Figure 6. Principal component analysis plots of molecular profiles from the bacterial deoxyribonucleic acid-positive samples. Each symbol is a representative of a single sample and is generated based on the data matrix derived from the band pattern of each sample. The symbols are plotted along the first two principal component (PC1 and PC2) axes. Percentage values at the axes indicate contribution of the principal components to the explanation of total variance in the dataset. The circles indicate three datasets obtained from the samples of the patients with different severity.

no significant difference in the prevalences of the bacteremia from the culture method in these patients between 4 or 5 days and 9 or 10 days (data not shown). Twenty-six isolates were identified from the positive blood cultures. *E. coli* grew in the cultures ($n = 10$, 10.4%) followed by *S. flexneri* ($n = 4$, 4.2%), *K. pneumoniae* ($n = 4$, 4.2%), *E. faecium* ($n = 3$, 3.1%), *S aureus* ($n = 3$, 3.1%), and *P. aeruginosa* ($n = 2$, 2.1%) (**Fig. 7*C***). DNA fragments showed that these bacteria were isolated and identified in the blood specimens by the molecular techniques and displayed an elevated prevalence compared with cultivation methods ($p < 0.05$). These results indicated that bacterial DNA-dependent molecular techniques were able to detect nonviable bacteria that could not be grown in cultures.

DISCUSSION

In this study, we have applied 16S rDNA-based molecular techniques to follow the prevalence of the bacteremia and the specific species of bacteria translocated into the systemic circulation in patients with AP. We demonstrate a high prevalence of bacteremia in patients with AP, with most of them being polymicrobial flora. The current data provide evidence that the bacterial species originate from the gut. Furthermore, the presence of circulating bacteria and their specific distribution are probably dependent on the severity of pancreatitis.

Bacterial translocation from the gut has been considered a central mechanism underlying the development of the pancreatic infections and necrosis (3–5, 33). Clinical and experimental studies have suggested that systemic infections in AP are probably caused by transmural migration of enteric microorganisms (3–7). Bacterial translocation has been proven to be a major player in acute inflammation and septic shock in AP (3–5). Therefore, early diagnosis of bacterial translocation and determination of bacterial species would contribute to reduce sepsis in patients with AP. Blood culture suffers from a lack of sensitivity and often fails to yield positive results even in the presence of infected pancreatic necrosis (19). In this study, we have developed 16S rDNA-dependent techniques to examine the prevalence of bacteremia in patients with AP. The findings show that the bacteremia is developed in 68.8% of the patients, which is far higher than the result

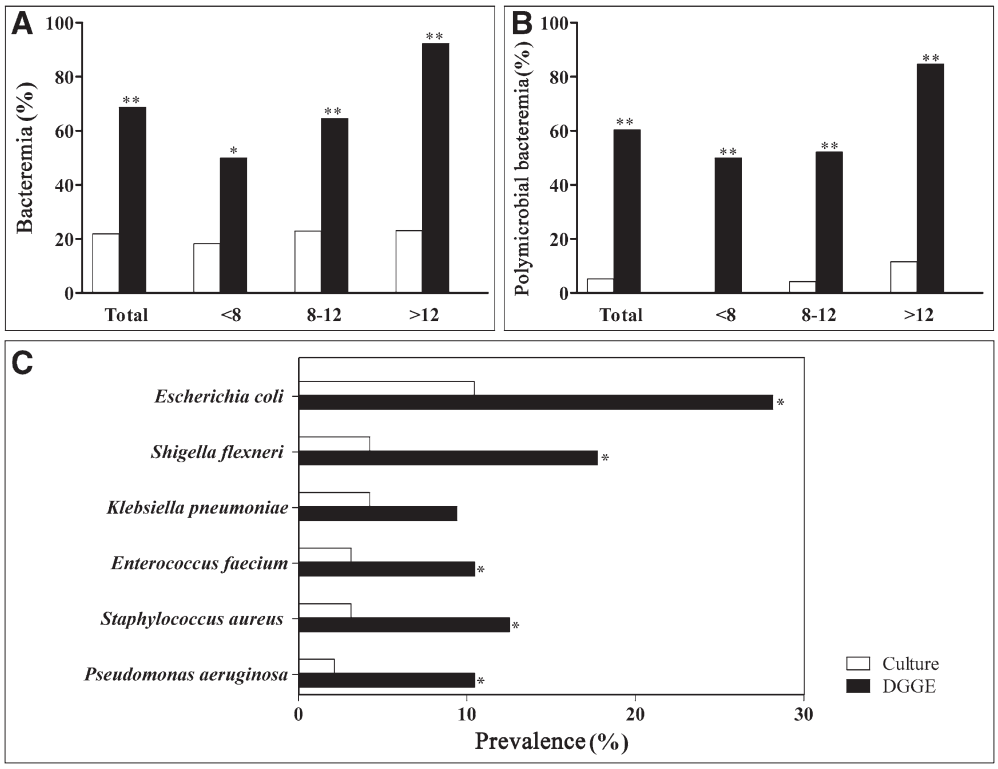

Figure 7. Comparison of denaturing gradient gel electrophoresis (DGGE) detection and conventional culture method for detecting microorganisms presented in the systemic circulation in patients with acute pancreatitis (AP). **A**, The variances of bacterial presence in peripheral blood in the patients with different severity. **B**, Molecular detection of DGGE showing higher frequency of polymicrobial bacteremia than that of conventional culture methods. **C**, The prevalence of bacteria in patients with AP as revealed by sequencing analysis and cultivation techniques $^{*}p < 0.05$, $^{**}p < 0.01$, compared with culture methods. *White bar* = culture, *black bar* = DGGE.

(21.9%) based on conventional culture methods (Fig. 7*A*). And the prevalence of the bacteremia is strikingly altered with the severity of the disease, as revealed by 92.3%, 64.6%, and 50.0% in the patients with greater than 12 points, 8–12 points, and mild AP, respectively. However, only 23.1%, 22.9%, and 18.2% of these patients are determined the presence of the bacteremia by cultivation methods (Fig. 7A). The presence of microorganisms in systemic circulation correlates with APACHE-II scores of the patients (Fig. 2*B*), suggesting that the bacteremia is significantly linked to increased severity of AP. We speculate that the worsening of the pancreatitis might promote further passage of bacteria from the gut lumen and lead to a self-perpetuating septic state especially in patients with SAP. In addition, more than half (60.4%) of the blood specimens consist of multiple bacterial species based on the DGGE analysis (Fig. 3*C*), indicating the nature of polymicrobial bacteremia in AP. However, only a few (5.2%) of the patients are identified as having polymicrobial flora by blood culture (Fig. 7*B*). It is probable that bacteria may translocate into the systemic circulation but escape detection by standard culture techniques. Most importantly, the presence of multiple organisms in systemic circulation is associated with the disease severity (Fig. 3*D*). Invasion of polymicrobial flora into peripheral blood may play a crucial role in systemic infections and septic complication in AP.

Sequence analysis of individual DGGE bands enables us to identify bacterial species presented in blood specimens from patients with AP. Here, we show that the opportunistic pathogens originating from the gut, such as *E. coli*, *S. flexneri*, *Pseudomonas* species, *E. bacterium*, *A. lwoffii*, *B. coagulans,* and *E. faecium*, are predominant in patients with AP (Table 3). *E. coli* is the most common pathogen presented in patients with AP and usually considered an indigenous pathogen from the gut. *E. coli* could translocate into the systemic circulation through the impaired intestinal barrier and present in extraintestinal sites in patients with SAP (3, 34, 35). A preponderance of Gram-negative aerobic bacteria, including *Pseudomonas* species, *S. flexneri*, *E. bacterium*, *A. lwoffii,* and *K. pneumoniae*, is also presented (Table 3), and these organisms are of close concern to the bacteremia in patients with AP (36, 37). Bacterial translocation could be the initiating factor of systemic infection and lead to septic complication in AP. We document that translocated bacterial spectrum at the species level remarkably changes in the patients with different severity (Fig. 5). In the patients with SAP with an APACHE-II score greater than 12 points, *E. coli*, *S. flexneri*, *A. lwoffii*, *E. bacterium*, *B. coagulans*, and *E. faecium* are mainly of predominance, whereas these bacterial species are presented less in the patients with 8–12 points and mild AP. The reduced prevalences in these common organisms are possibly attributable to the clinical status of the patients.

Molecular detection of bacterial DNA in blood samples by PCR has been described in intestinal bacterial translocation (19–22). It has been reported that bacterial DNA in peripheral blood is detected as positive in 33.4% of patients with mild AP and in 43% of patients with SAP using PCR technique (21, 38). However, PCR failed to detect multiple microorganisms in a single specimen (22), which may limit its use in the assessment of polymicrobial flora. Using universal bacterial primers, unknown and/or uncultivable species in a complex microbial community can be amplified and then separated in the DGGE gels. In this study, we investigate the usefulness of DGGE and DNA sequencing in detecting bacteria circulated in the peripheral blood of patients with AP. Bacteria are detected in 68.8% of the samples from patients with AP, and most of these positive samples consist of polymicrobial flora (Figs. 1 and 3*C*). The data suggest that 16S rDNA-dependent molecular techniques provide an effective tool for the diagnosis of diverse organisms in the peripheral blood of patients with AP.

The administration of prophylactic antibiotics remains controversial in the management of AP (39–44). As the mortality of SAP is obviously associated with the complications of infection, the patients are often administrated prophylactic broad-spectrum antimicrobial agents with the aim of reducing the prevalence of pancreatic and peripancreatic infections (39, 40). The antibiotic prophylaxis is not beneficial for reducing the risk of developing infected pancreatic necrosis and nonpancreatic infection in many clinical trials (41–44). More recent randomized controlled trials (RCTs) do not support the benefit of prophylactic antibiotics (45–47), and meta-analyses on the topic offer conflicting recommendations (48, 49). In this study, we compare the difference in the prevalence of bacteremia at two different time points after prophylactic antibiotics are administrated for patients with AP. No significant differences in the prevalence of bacteremia in the patients with AP are observed between 4 or 5 days and 9 or 10 days (Fig. 2*A*), whereas the prevalence of polymicrobial bacteremia in SAP patients with greater than 12 points shows a trend toward to a decrease at the latter time point (Fig. 3*C*). Prophylactic antibiotic use might affect the results in the prevalence of bacteremia in AP to some extent, which is a major limitation of this study. It is difficult to prevent clinicians from administering antibiotics empirically to patients with AP. The prevalence of bacteremia presented here might be underestimated due to administration of routine broad-spectrum prophylactic antibiotics. And it could be increasing in AP without treatment of prophylactic antimicrobial agents. It needs to be further clarified by more high-quality RCTs. Our study was to elucidate a potential association between the prevalence of bacteremia and the disease severity of AP. Our conclusion on the relationship between the prevalence of the bacteremia and the disease severity of AP can be drawn despite the limitation of antibiotic prophylaxis.

Nevertheless, our findings suggest that DGGE technique is a superior tool to study the pathophysiology of bacterial translocation in AP. This study only detects the presence of bacteria in peripheral blood and does not investigate whether other intraperitoneal or extraperitoneal organs are also infected; therefore, it does not conclude about the route of infection and the existence of sepsis in patients. The questions above should be addressed in future studies.

In summary, we define the occurrence of the bacteremia and identify the characteristic of translocated bacteria in patients

with AP using 16S rDNA-based molecular approaches. We demonstrate an association between the bacteremia and the severity of AP. Elucidation of bacterial translocation would be a significant advance in understanding the pathogenesis of local and systemic infections in AP. It would also contribute to developing a novel strategy for the prevention and treatment of septic complication.

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