0031-3998/05/5801-0143
PEDIATRIC RESEARCH
Vol. 58, No. 1, 2005
Printed in U.S.A.

Rapid Diagnosis of Bacterial Sepsis with PCR Amplification and Microarray Hybridization in 16S rRNA Gene

SHIQIANG SHANG, GUOXIAN CHEN, YIDONG WU, LIZHONG DU, AND ZHENGYAN ZHAO

Lab Center [S.S., Y.W.], Department of Neonatology [L.D., Z.Z.], Affiliated Children's Hospital of Medical College, Zhejiang University, Hangzhou 310003, P.R. China, Department of Burn Injuries [G.C.], The Second Affiliated Hospital of Medical College, Zhejiang University, Hangzhou 310009, P.R. China

ABSTRACT

In this study, blood culture and PCR-microarray analysis were used to examine 172 cases of suspected septicemia. Primers and oligonucleotide probes, based on the sequences of bacterial 16SrRNA gene, were arrayed by imprinting on microarray slides. Blood specimens collected from 172 cases of suspected septicemia were cultured and then tested separately by PCR for the bacterial 16S rRNA. Of the 172 clinical cases, 17 cases tested positive by PCR. The number of positives identified by PCR (9.88%) was significantly higher than the number of positives identified by the blood culture (4.65%). When blood culture was used as control, the sensitivity of PCR was 100%, the specificity was 97.85%, and the index of accurate diagnosis was 0.979. When the 17 PCR positive specimens were further analyzed by hybridization against the microarrays, five were found to be probe positive for *E. coli*, four were positive for S. epidermidis, four were positive for CoNS, and two were positive for *Bacillus* and Propionibacterium, respectively. In the eight specimens showing positive results by both PCR and blood culture, the species determined by microarray analysis corresponded with the result obtained from blood culture. Detection of the bacterial 16SrRNA genes in clinical specimens by PCR and microarray analysis can be used to accurately diagnose neonatal sepsis. This method has a higher sensitivity and specificity than blood culture and can provide a rapid way for the etiological diagnosis of neonatal septicemia. **(*Pediatr Res* 58: 143–148, 2005)**

Abbreviations

***C. neoformans*,** Cryptococcus neoformans
CMV, cytomegalovirus
CoNS, coagulase-negative Staphylococcus
EBV, Epstein-Barr virus
HBV, hepatitis B virus
LOS, late-onset sepsis
NICU, neonatal intensive care unit,

Evaluation of patients with the signs and symptoms of bacterial sepsis requires a combination of clinical acumen and laboratory support. The diagnosis of bacterial infection can be particularly difficult in certain patients, such as neonates and young infants. Blood culture is widely used for the diagnosis of septicemia. However, in many clinical situations the yield from blood culture is low, positive cultures are obtained from fewer than 30%. Some of the patients with false-negative blood culture may have had prior antibiotic treatment or they were not bacteremic at the time of blood collection. In recent years, the sequence of the 16S rRNA gene has been used to detect and identify bacterial infection in clinical practice (1), but many of the PCR methods in use are not able to further differentiate between different bacterial species. DNA microarrays offer a means of enhancing the detection and identification capabilities of PCR. In this report, microarrays serve as a series of parallel dot-blots for rapid detection of sequence variation within the 16S rRNA gene and for detection of the products resulting from the PCR using conserved primers in the 16S rRNA gene. One hundred and ten strains of standard bacteria and corresponding clinical isolates were amplified by PCR and their products were further analyzed by DNA microarrays. Finally, we applied PCR amplification and microarray hybridization to assess clinical bacterial infection. We hypothesized that the PCR-microarray proves to be the most effective way of detecting bacteria in clinical practice and that it cannot only discover the existence of bacteria, but also identity the species and guide the clinical antibiotic selection.

Received August 4, 2004; accepted November 15, 2004.
Correspondence: Shiqiang Shang, M.D., Lab Center, Affiliated Children's Hospital of Medical College, Zhejiang University, Hangzhou 310003, China; e-mail: shangsq33@mail.hz.zj.cn
The study was supported by grants from Provincial Natural Science Foundation of China (No. 398426 and No. 396457) and partially supported by a grand of the Provincial Great Science Foundation of China, Grant No. 001103059–01

DOI: 10.1203/01.PDR.0000169580.64191.8B

MATERIALS AND METHODS

Bacterial strains and clinical specimens. The 110 strains, including 26 genus and 48 species, used in this study are listed in Table 1. For negative

Table 1. *Number of strains studied for each species/genus and results of PCR and microarrays*

				Microarray hybridization				
Genus	Species	Number	PCR	Universal 1	Universal 2	Gram +	Gram −1	Gram −2
Acinetobacter	*A. Iwoffi**	1	+	+	+	−	+	+
	A. calcoacaticus	4	+	+	+	−	+	+
Aeromonas	*A. hydrophila**	1	+	+	+	−	+	+
Alcaligenes	*A. faecalis*	2	+	+	+	−	+	+
Bacteroides	*B. Fragilis*	1	+	+	+	−	+	+
Citrobacter	*C. Freundil**	1	+	+	+	−	+	+
Edwardsiella	*E. tarda*	1	+	+	+	−	+	+
Enterobacter	*E. aerogenes**	1	+	+	+	−	+	+
	E. cloacae	3	+	+	+	−	+	+
Escherichia	*E. coli**	7	+	+	+	−	+	+
Flavobacterium	*F. meningosepti*	1	+	+	+	−	+	+
Haemophilus	*H. ducreyi*	1	+	+	+	−	+	+
	*H. influenzae**	1	+	+	+	−	+	+
Klebsiella	*K. pneumoniae**	8	+	+	+	−	+	+
Neisseria	*N. meningitides*	1	+	+	+	−	+	+
Proteus	*P. mirabilis**	1	+	+	+	−	+	+
	P. vulgaris	1	+	+	+	−	+	+
	P. penneri	3	+	+	+	−	+	+
Providencia	*P. stuartii*	1	+	+	+	−	+	+
Pseudomonas	*P. aeruginosa*	5	+	+	+	−	+	+
	P. putida	3	+	+	+	−	+	+
Burkholderia	*B. cepacia*	1	+	+	+	−	+	+
Salmonella	*S. typhimurium**	1	+	+	+	−	+	+
	*S. enteritidis**	1	+	+	+	−	+	+
Serratia	*S. marcescens**	3	+	+	+	−	+	+
Yersinia	*Y. enterocolitica*	1	+	+	+	−	+	+
Bacillus	*B. subtilis**	3	+	+	+	+	−	−
Corynebacterium	*C. pseudotuberculosis*	1	+	+	+	+	−	−
Listeria	*L. monocytogenes*	1	+	+	+	+	−	−
Micrococcus	*M. bovis*	1	+	+	+	+	−	−
Mycobacterium	*M. tuberculosis*	2	+	+	+	+	−	−
Propionibacterium	*P. acnes*	1	+	+	+	+	−	−
Staphylococcus	*S. aureus**	9	+	+	+	+	−	−
	*S. epidermidis**	11	+	+	+	+	−	−
	S. capitis	1	+	+	+	+	−	−
	S. cohnii	1	+	+	+	+	−	−
	S. haemolyticus	6	+	+	+	+	−	−
	S. hominis	3	+	+	+	+	−	−
	S. saprophyticus	1	+	+	+	+	−	−
	S. warneri	2	+	+	+	+	−	−
	S. sciuri	1	+	+	+	+	−	−
Streptococcus	*S. agalactiae**	3	+	+	+	+	−	−
	S. bovis	1	+	+	+	+	−	−
	S. mitis	1	+	+	+	+	−	−
	*S. pneumoniae**	4	+	+	+	+	−	−
	*S. pyogenes**	1	+	+	+	+	−	−
	S. salivarious	1	+	+	+	+	−	−

Note: * showed that one of them is ATCC or NCTC.

controls, we used total human DNA, cytomegalovirus (CMV), hepatitis B virus (HBV), EBV (EBV) and *Cryptococcus neoformans (C. neoformans)*. One hundred seventy-two blood specimens from patients with suspected bacterial infections (sepsis and/or meningitis) (2) were evaluated by routine bacterial culturing and PCR-microarray hybridization. The study was approved by the Medical Ethics Committee of the Medical College, and informed consent was obtained. During the study period from January 1, 2004 to June 30, 2004, we included all neonates who were hospitalized in our neonatal ward and the NICU and developed clinical signs suggestive of sepsis after 3 d of life. These signs included: fever, temperature instability, apnea, dyspnea, cyanosis, respiratory distress, vomiting, abdominal distension, lethargy, irritability, hypoglycemia, hyperglycemia and excessive hyperbilirubinemia. In addition, 30 blood specimens from healthy neonates served as negative controls. The collection of blood samples from healthy children was done during the physical examination before entering kindergarten, which was approved by the provincial medical ethics committee. Bacteria were detected by culture of blood and PCR testing.

Primers and oligonucleotide-probe set. Eighteen oligonucleotide probes of 25–28 nucleotides in length, targeting the bacterial 16S rRNA, were selected from the rRNA database (http://www.ncbi.nlm.nih.gov). They included two universal probes, one Gram-positive probe, two Gram-negative probes, and other specific probes (Table 2). We also designed a pair of highly conserved primers based on a computer alignment of ribosomal RNA sequences available through GenBank. The sequence of the primers was as follows: upstream 5'-TGCGGTTGGATCACCTCCT-3', downstream 5'-TCCCCACCTTCCAGTT-3'.

DNA isolation. All bacterial strains were treated in 400 μL of 5% Chelex100 buffer (containing 0.03% SDS, 1% Tween-20 and 1% NP40) and boiled for 15 min. DNA of each sample was collected and amplified from the supernatant. To lyse *Staphylococcus*, proteinase K was added to the 5%

Table 2. *Oligonucleotide probes used in this study and their specificities*

Number Location	Specificity		Sequence (5′-3′)
	Length (nt)	GC (%)	
1 Universal 1			5′-CGGTCGTGTAGGTTGGCCAGCCACTTA-3′
1369–1395	27	52	
2 Universal 2			5′-CGGTCGTGTAGGTTGGCCAGCCACCCTG-3′
1369–1396	28	68	
3 Gram-positive			5′-CAGCACTTAAGTACTACGGGCTTATGTC-3′
1088–1115	28	46	
4 Gram-negative 1			5′-CTGCTAAGCCGCATGATGTGAAGACGTC-3′
1190–1217	28	54	
5 Gram-negative 2			5′-CTGCTAAGCCGCAGGATGTGAAGACGTC-3′
1190–1217	28	57	
6 *S. aureus*			5′-CGCGCAGCACTAACCTTTTACCACG-3′
1435–1459	25	52	
7 *Escherichia coli*			5′-CGGCGTTACGACTTTCTCTAACATG-3′
1458–1482	25	48	
8 *S. pneumoniae*			5′-TTCAGAGACACCGTTTATGAGTTAA-3′
1278–1302	25	36	
9 *S. agalactiae*			5′-TAAACACAATTTGCGAATCTCAGAT-3′
1278–1302	25	32	
10 Neisseria meningitides			5′-ATGCCGCCACGCGGTCGGTAACT-3′
1261–1283	23	58	
11 Haemophilus species			5′-GGAGTGCGTTCTAGCGAGAAGTAGAT-3′
1416–1441	26	50	
12 Listeria monocyto genes			5′-GGAGTGCGTTCTAGCGAGAAGTAGAT-3′
1277–1301	25	32	
13 Bacteroides-Flavobacterium			5′-GAGCTTAGGCCCGTGCTGTAATGACGTC-3′
1190–1217	28	57	
14 *S. epidermidis*			5′-GCACGGCATCGTCCAATTGGTTACT-3′
1443–1467	25	52	
15 CoNS			5′-CGCGTACGTCCAAATGGTTACTCTA-3′
1440–1464	25	48	
16 Bacillus			5′-CATAAGAGCCCGGCATGCTGATCCG-3′
1354–1378	25	60	
17 Corynebacterium			5′-ACTCATCCGACGTAAGTAGCATGTC-3′
1228–1252	25	48	
18 Propionibacterium			5′-GCTGTGATCCAAGGCCCGGGAACGTA-3′
1376–1400	25	60	

Chelex100 buffer, and the mixture was incubated at 56°C for 60 min and then boiled for 15 min. Blood samples in the presence of EDTA were subjected to red cell lysis in five volumes of 0.87% NH_4Cl, leukocytes were pelleted, suspended in 200 μL of 5% Chelex100 buffer with 20 mg/mL proteinase K, incubated at 56°C for 60 min and then boiled for 15 min, centrifuged for 1 min, and the supernatant was collected for PCR amplification.

PCR amplification. The PCR mixture (50 μL) contained 50 mM Tris-HCl (pH 9.0), 50 mM KCl, 7 mM $MgCl_2$, 2 mg/mL BSA, 16 mM $(NH_4)_2SO_4$, 100 μM (of each) primer, and 1.0 U of *Taq* polymerase (Boehringer, Inc., Mannheim, Germany). The PCR mixture was incubated with 0.5 μg of DNase I, which is active on double-stranded DNA (dsDNA, Boehringer, Mannheim, Inc., Germany) for 15 min at room temperature. The DNase was inactivated by incubating the mixture for 10 min at 95°C. The 5 μL of the cell lysates, which contained the target DNA, was added to the PCR mixture. PCR was performed for 32 cycles of 1 min at 94°C, 15s at 55°C and 45 s at 72°C on a DNA thermocycler (Perkin-Elmer Cetus, Inc., Norwalk, CN). After amplification, the samples were incubated for another 5 min at 72°C.

Preparation of glass slides and spotting. The DNA microarray format used in this study is based on standard microscopic glass slides (CEL, Inc., USA). Slides were activated by 1,4-phenylenedisothiocyanate treatment for covalent binding of 5'-amino-modified capture oligonucleotides. Probes were spotted onto the activated slide surface with the spotting device robodrop (Cartesian, Inc., USA). The concentration of the amino-modified oligonucleotides in PCR-water was 10 μM with 1% glycerol. Volumes of deposited probe solutions were about 250 pL, resulting in spots with a diameter of approximately 200 μm. To complete covalent binding, after being spotted with probe solutions, slides were incubated at room temperature for 2 h in a wet chamber to prevent the evaporation of the spots. Blocking of the slides was performed in 6-amino-1-hexanol (50 mM) and diisopropylethylamine (150 mM) in dimethylformamide. Finally, the slides were washed with deionized, particle-free water, air dried, and stored under nitrogen at 4°C.

DNA microarray hybridization. Hybridization and washing of the microarrays were performed according to a standard protocol from the ArrayIt Handbook (http://www.arrayit.com). For hybridization to the array, we combined 8 μL of the PCR products with 8 μL of 5×SSPE, 1%SDS to a final volume of 16 μL. This mixture was placed in a boiling water bath for 2 min, cooled on ice, then applied to the surface of a blocked and immobilized microarray that had been prewarmed to 48°C. The microarray and hybridization were overlayed with a coverslip and transferred to a prewarmed chamber that was kept humid by enclosing a folded paper towel moistened with 2×SSC. The array was incubated in 48°C for 1/2h. Then the array was washed at room temperature, first in 2×SSC, 0.01%SDS for 2min, then in 0.1×SSC, 0.01%SDS for 3min and finally in 0.01%SDS for 1min. Slides were dried at room temperature. Unless otherwise specified, probes were applied for hybridization experiments in the standard way, i.e., all probes immobilized *via* their 5' end and at the same concentration (10 μM). For reducing steric hindrance capture probes carrying polyadenosine triphosphate spacers of 6, 9, 12, or 15 nucleotides, located at the 5' end of the capture oligonucleotide, were used.

Signal detection and data analysis. Air-dried slides were imaged at a resolution of 10 μm with a ScanArray Express microarray scanner (PerkinElmer, Inc., Fremont, CA) using the same laser power and sensitivity level of the photomultiplier for each slide. Therefore, absolute signal intensities (arbitrary units) obtained from independent experiments should be directly comparable. For automatic spot detection and signal quantification, the ScanArray Express software was used. Filters for the automatic removal of spots with poor circularity or low uniformity of pixel intensities were established. Signals were considered positive if values were above zero after local background correction. This strategy for assessing the reliability of positive spots

was possible because nonspecific binding of labeled target molecules to the slide surface was not observed for regions where oligonucleotides were located.

Blood culture. A total of 0.5 to 1.0 mL of blood was obtained from 172 selected patients with suspected septicemia by venipuncture under sterile conditions and inoculated into 20 mL of tryptone soya broth or thioglycolate broth for aerobic and anaerobic culture of organisms. The cultures were incubated at 35°C and inspected visually every morning. Subcultures were performed 14 to 18 h after inculcation. Samples were removed from each culture bottle aseptically with a sterile 1 mL syringe, and one drop was subcultured on duplicate blood agar and chocolate agar, which were then incubated at 35°C. One blood agar plate, along with the chocolate agar, was incubated in 5% CO_2. The positive growth was recorded and biochemical tests were carried out to identify the bacteria from blood culture at 48 h to 72 h.

RESULTS

Specificity of universal primers and sensitivity of the PCR assay. To verify that the primers used in this study were specific for bacteria (110 species), human DNA, CMV, HBV, EBV and *C. neoformans* were tested. Amplified products of 371 bp were detected from bacterial DNA, but not from human DNA, CMV, HBV, EBV, and *C. neoformans*. Therefore, our primers did not cross-react with DNA from human, viruses and fungi, and were highly specific for bacteria. The sensitivity of detection of amplification with the universal primers was examined. Serial 10-fold dilutions of *E. coli* DNA were prepared and added to the reaction mixture. At 32 cycles, it was possible to reproducibly detect a band using concentrations equal to 1 pg of the *E. coli* 16S rRNA gene, which corresponds to the amount of DNA contained in approximately three organisms (3)**.**

General steric hindrance. Poly (T) spacers of different lengths (none, 3-mer, 6-mer, 9-mer, 12-mer, and 15-mer) were tested on universal probe 1. Oligonucleotide probes with polydT spacers of 0, 3, 6, 9, 12, or 15 nucleotide were synthesized and attached to slides. The hybridization signal was virtually nonexistent for polydT spacers of 3-mer or less, but then increased strongly with length up to 9-mer and kept higher signal from 9-mer to 15-mer. We selected 9-mer spacer as general spacer molecules.

Results of 16S rRNA gene PCR and bacterial culturing. Of 172 specimens with suspected septicemia, 17 (9.88%) were positive by PCR, while 8 specimens (4.65%) were positive by blood culture. The positive rate of PCR was significantly higher than that of blood culture ($p < 0.01$) (Table 3). Thirty blood samples from healthy children were confirmed to be negative by both blood culture and PCR. When blood culture was used as a control, the sensitivity of PCR was 100%, the specificity was 97.85%, and the index of accurately diagnosis was 0.979.

Microarray hybridization testing. The amplified products of expected size (371bp) were detected in 110 strains, including 55 Gram-positive and 55 Gram-negative strains. Using the amplified DNA of *55 Gram-positive bacteria as templets, there were positive findings for the two universal probes and the one Gram-positive probe*. Two Gram-negative probes and two universal probes showed a positive result when 55 Gram-negative bacteria were used as templets. Each strain specifically hybridized with its own probe (Fig. 1–3).

The seventeen specimens with positive results by PCR were further hybridized on microarrays. All were positive by universal probes. Of those 17 specimens, five were positive by *E. coli* probe, four by *S. epidermidis*, four by CoNS, two by *Bacillus* and Propionibacterium, respectively. For the eight specimens positive by both PCR and blood culture, the species determined by microarray analysis corresponded to that determined by traditional blood culture (Table 4).

Table 3. *Results obtained with clinical sample by PCR and blood culture*

		Blood culture		
		Yes	No	Total
PCR	Yes	8	9	17
	No	0	155	155
Total		8	164	172

$\chi^2 = 178.09$; $p < 0.01$.

DISCUSSION

Bacterial pathogens pose a significant threat to human health. Consequently, considerable effort has been devoted to the development of rapid, sensitive, and specific assays for the

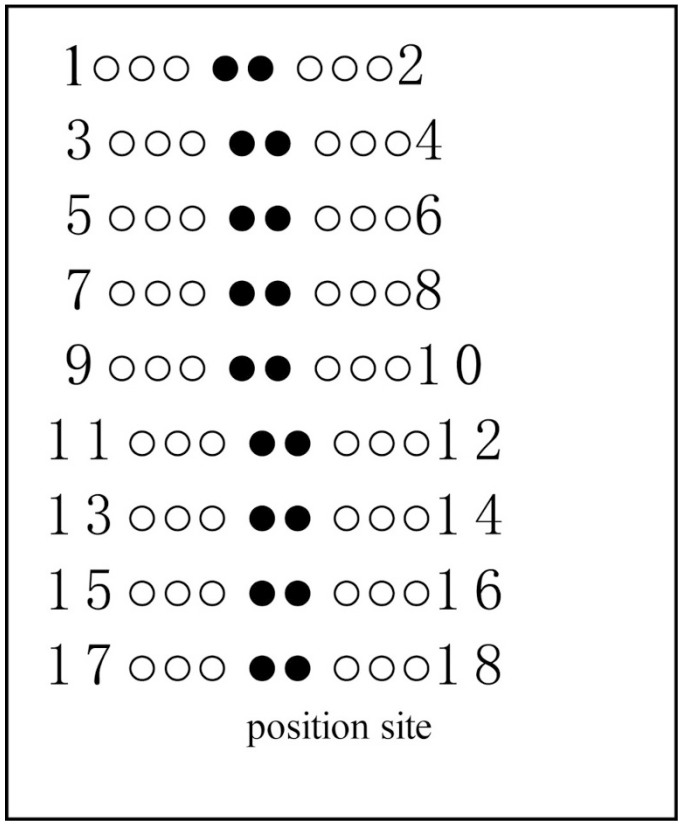

Figure 1. Image of probe position with a scanned microarrays. The black circles (two middle columns) serve as position sites (orientation points). The blank circles indicate the positions where different bacterial probes were printed in replicate (3 replicate circles on line for one exact probe). All probes on the array include line one for Universal probe 1, two for Universal 2, three for Gram-positive, four for Gram-negative 1, five for *S. aureus*, six for Gram-negative 2, seven for *S. epidermidis*, eight for *E. coli*, nine for CoNS, ten for *Haemophilus* species, 11 for *L. monocytogenes*, 12 for *S. pneumoniae*, 13 for S. agalactiae, 14 for *Bacteroides-Flavobacterium*, 15 for *Bacillus*, 16 for N. meningitides, 17 for Corynebacterium, and 18 for Propionibacterium.

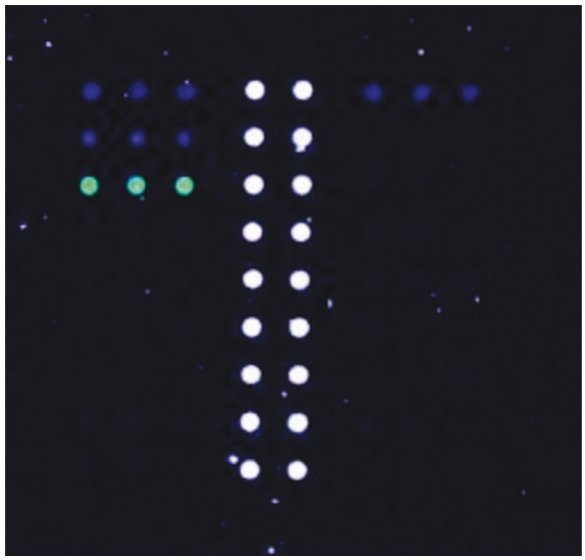

Figure 2. The result of microarray hybridization of *S. aureus* showed in Fig. 2. Visible spots are located in line 1 (Universal probe 1), line two (Universal 2), line three (Gram-positive) and line five (*S. aureus*),

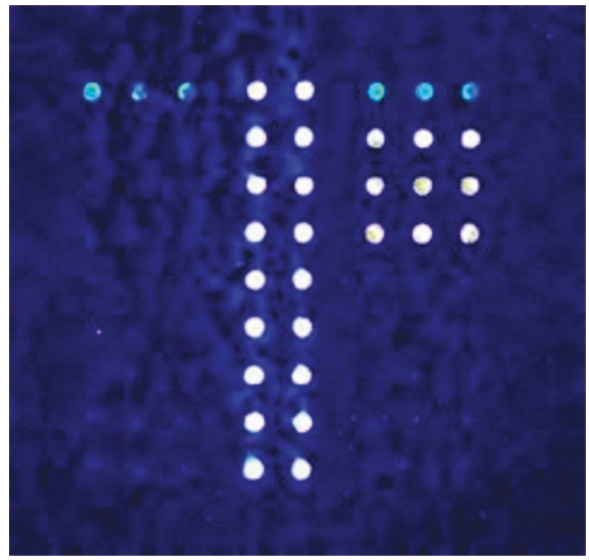

Figure 3. The result of microarray hybridization of *E. coli* showed in Fig. 3. Visible spots are located in line 1 (Universal probe 1), line two (Universal 2), line four (Gram-negative 1), line six (Gram-negative 2) and line eight (*E. coli*).

detection of these organisms. PCR is the most important molecular tool because of the potential for detecting less than 3 copies of a specific bacterium (1). Conventional PCR assays incorporate a pair of oligonucleotide primers to amplify a specific gene that is then detected using agarose gel electrophoresis combined with an intercalating dye (e.g., ethidium bromide) and UV light. If necessary, southern blots, dot-blots, or sequencing can be used to positively identify specific PCR products. The concept of DNA microarray hybridization was introduced recently, and the technique has been applied for a few years in clinical diagnosis and many fields of research, e.g., functional genomics and genetic analysis. In contrast, DNA microarrays are still not common in microbial diagnosis. Microarrays can be coupled with PCR where they serve as a set of parallel dot-blots to enhance products detection and identification (4–8). Microarray can be used to identify bacterial strains via reverse hybridization, as we have done it before (1). Microarrays are composed of many discretely located probes on glass. Each probe is composed of a sequence that is complimentary to a specific gene sequence. PCR is used to amplify DNA and the products are then hybridized to the array to identify different specific DNA.

In a previous study (1), we have used the 16S rRNA gene to detect bacterial infection in neonatal septicemia. The method showed excellent specificity and sensitivity when applied to identify bacterial strains. In this study, we continued using the prior primers and amplified a 371 bp DNA fragment from 110

Table 4. *PCR microarrays and blood culture results obtained with 17 blood samples*

Case No.	Gender	Gestational age	Birth weight	Age at LOS (days)	Blood culture	PCR	Microarray			
							Universal	Gram +	Gram −	Specific
1	M	29	1390	11	*S. epidermidis*	+	+	+	−	*S. epidermidis*
2	M	31	1410	8	*S. epidermidis*	+	+	+	−	*S. epidermidis*
3	M	28	1290	9	*S. epidermidis*	+	+	+	−	*S. epidermidis*
4	F	33	1950	13	*S. epidermidis*	+	+	+	−	*S. epidermidis*
5	F	38	2690	15	*S. hominis*	+	+	+	−	CoNS
6	M	29	1300	10	*S. haemolyticus*	+	+	+	−	CoNS
7	M	30	1310	7	*S. sciuri*	+	+	+	−	CoNS
8	M	37	2560	9	*E. coli*	+	+	−	+	*E. coli*
9	M	38	3100	10	−	+	+	−	+	*E. coli*
10	F	40	3010	7	−	+	+	−	+	*E. coli*
11	M	39	3200	17	−	+	+	−	+	*E. coli*
12	F	39	2980	6	−	+	+	−	+	*E. coli*
13	M	31	1860	7	−	+	+	+	−	CoNS
14	M	32	1970	4	−	+	+	+	−	Bacillus
15	F	34	2100	9	−	+	+	+	−	Bacillus
16	M	30	1410	14	−	+	+	+	−	Propionibacterium
17	F	30	1470	18	−	+	+	+	−	Propionibacterium
Total					8	17	17	12	5	17

Note: +: positive; −: negative.

different types of bacteria. The primers did not cross-react with human or viral DNA. Blood specimens from 172 cases of suspected septicemia were cultured and detected bacterial 16S rRNA separately. The results of our study showed that the sensitivity of PCR for the detection of bacterial sepsis was higher than that of traditional blood culture. This could be due to the fact that PCR amplification can allow for detection of bacterial levels blow the limit of blood culture, particularly in patients treated with antibiotics. Microarrays allow for multiple probes to be used on a single sample, so that microarray hybridization can detect and differentiate among multiple bacterial strains in clinical samples. We designed three series of oligonucleotide probes to detect bacterial PCR products. The first series of probes was broad in range and consist of two universal probes, a Gram-positive probe, and two probes for Gram-negative species. The second series was designed to distinguish seven major bacterial species frequently causing sepsis, including *Neisseria meningitides, Haemophilus influenzae, Streptococcus pneumoniae, S. agalactiae*, *E. coli, Listeria* monocytogenes, and *S. aureus*. The third series was designed for the detection of commonly contaminants, including *Bacillus*, Corynebacterium, Propionibacterium, *Bacteroides-Flavobacterium*, and CoNS. Our PCR primer/microarray probe system was able to correctly diagnose all 110 bacterial strains.

The 17 specimens positive by PCR were further hybridized on microarrays. All were positive by universal probes and were recognized as specific cells. All 17 cases positive by PCR were also positive by blood culture with both methods identifying the same bacterial species. CoNS and P. acnes are pathogens of low virulence and usually infect the patients with impaired immune function or premature infants. Of 17 PCR positive cases, eight were positive to CoNS (7 were positive and one was negative with blood culture), two cases that belonged to negative with blood culture were positive for P. acnes. CoNS was reported to be the most common case of neonatal sepsis in China. VLBW and presence of central venous catheters or other foreign bodies are risk factors associated with the development of invasive CNS infection in the USA, but in China, where central venous catheters or other foreign bodies are not often used in neonates. Our results showed that, of 8 CoNS positive cases, 7 were premature infants and one was low-birth-weight-infant (Table 4). Just only two cases (No.3 and No.6) had *indwelling foreign devices* (both are i.v. catheters). It could be seen that low virulence organisms may be common infectious causes in premature infants and/or low-birth-weight infants. The microarray detection method described here can be completed within 6 h or less, which is significantly less than the time required for traditional blood culture. Thus, the 16Sr-RNA PCR with microarray detection method makes it possible to use a single blood sample to differentiate among over 100 bacterial strains and provides a more sensitive means of detecting bacteria in clinical specimens including those from neonates.

REFERENCES

1. Shang S, Chen Z, Yu X 2001 Detection of bacterial DNA by PCR and reverse hybridization in the 16S rRNA gene with particular reference to neonatal septicemia. Acta Paediatr 90:179–183
2. Yu JL, Wu SX 2003 Diagnosis and treatment of neonatal bacterial sepsis. Chinese J Pediatr 41:897–899
3. Shirai H, Nishibuchi M, Ramamurthy T. Bhattacharya SK, Pal SC, Takeda Y 1991 Polymerase chain reaction for detection of the cholera enterotoxin operon of vibrio cholerae. J Clin Microbiol 29:2517–2521
4. Bischoff M, Dunmam P, Kormanec J, Macapagal D, Murphy E, Mounts W, Berger-Bachi B, Projan S 2004 Microarray-based analysis of the Staphylococcus aureus sigma B regulon. J Bacteriol 186:4085–4099
5. Meloni R, Khalfallah O, Biguet NF 2004 DNA microarrays and pharmacogenomics. Pharmacol Res 49:303–308
6. Hinton JC, Hautefort I, Eriksson S, Thompson A, Rhen M 2004 Benefits and pitfalls of using microarrays to monitor bacterial gene expression during infection. Curr Opin Microbiol 7:277–282
7. Bodrossy L, Sessitsch A 2004 Oligonucleotide microarrays in microbial diagnostics. Curr Opin Microbiol 7:245–254
8. Bryant PA, Venter D, Robins-Browne R, Curtis N 2004 Chips with everything: DNA microarrays in infectious disease. Lancet Infect Dis 4:100–111