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DNA microarray-based detection of nosocomial pathogenic *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

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Abstract

Infection by nosocomial pathogenic bacteria is increasingly becoming a major threat to the patients in the hospital. We have developed a diagnostic DNA microarray for the detection of two important nosocomial pathogens, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The diagnostic DNA microarray contains the species-specific probes of 15mer oligonucleotides designed based on the sequences of 23S ribosomal DNA. The performance of DNA microarray in diagnosing *P. aeruginosa* and *A. baumannii* was evaluated using reference bacteria as well as clinical specimens such as blood, stool, pus, sputum, urine and cerebrospinal fluid. Using this DNA microarray, *A. baumannii* could be successfully detected in 11 out of 13 clinical specimens, thus giving the sensitivity of 84.6% with the specificity of 100% and the positive predictive value of 100%. *P. aeruginosa* could also be detected in 25 out of 26 clinical specimens, showing the sensitivity of 96.2%, the specificity of 100%, and the positive predictive value of 100%. These results suggest that two nosocomial pathogens, *P. aeruginosa* and *A. baumannii*, can be efficiently diagnosed by using the DNA microarray developed in this study.

Keywords: Nosocomial pathogen; *P. aeruginosa*; *A. baumannii*; DNA microarray

1. Introduction

Nosocomial infections have affected 5% of all patients admitted to hospitals, resulting in 88,000 deaths and accounting for 4.5 billion dollars in health care costs annually [1]. The control of hospital-acquired infections caused by antibiotic-resistant gram-negative bacilli has become an important mission over the last 20 years in developed countries. These gram negative bacilli have been implicated in a variety of nosocomial infections including pyelitis, cystitis, meningitis, injury infection, endocarditis, skin and wound sepsis, respiratory tract and urinary tract infection, and severe community-acquired and nosocomial pneumonia. Among the nosocomial pathogens, *Pseudomonas aeruginosa* has emerged in recent years as one of the most important pathogenic bacteria and a leading cause of bacteremia having morbidity and mortality rate ranging from 25–50% [2]. *Acinetobacter baumannii* is an etiological agent of bacteremia and nosocomial pneumonia having mortality rate of greater than 70% [3–6]. It is an opportunistic pathogen associated with wound infections, urinary tract infections, and may be found to contaminate medical equipments for inhalation and dialysis. Due to their clinical importance, it is urgently needed to develop a simple yet accurate method to diagnose them.

It is becoming important to increase the sensitivity and specificity of diagnostic routines and to correctly identify the nosocomial pathogens. Among such methods are the DNA restriction fragment length polymorphism (RFLP) analysis determined by pulsed-field gel electrophoresis [7], repetitive

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extragenic palindromic polymerase chain reaction (REP-PCR) [8], fingerprinting technique [9], universal PCR coupled with restriction enzyme analysis [10], and analysis using monoclonal antibodies specific for the *o*-polysaccharide moiety of the lipopolysaccharide (LPS) [11]. These methods have their own pros and cons. Especially, PCR method has been frequently adopted due to its higher speed, sensitivity and specificity for identifying pathogenic bacteria. However, there is a major problem of false positive amplifications caused by either sequence variations in the primer binding sites of the target DNA region or low sensitivity. DNA microarray-based assay recently developed is a simple yet powerful method that allows simultaneous detection of multiple targets using less sample volume [12]. Since, it provides additional advantages of enhanced accuracy and reduced assay time, it can be employed to detect pathogenic bacteria if the species-specific probes are available [13]. Unfortunately, the specific probes for the detection of *P. aeruginosa* and *A. baumannii* have not so far been reported.

Here, we report the development of DNA microarray for detecting two nosocomial pathogens, *A. baumannii* and *P. aeruginosa,* using the species-specific 15mer oligonucleotide probes designed based on the sequences of the 23S ribosomal DNA (rDNA) from public databases (for *P. aeruginosa*) and those we determined (for *A. baumannii*). We also report the results of DNA microarray-based assays using various specimens such as blood, stool, pus, sputum, urine and cerebrospinal fluid (CSF).

2. Materials and methods

2.1. Bacterial strains

Reference bacterial strains were obtained from the American Type Culture Collection (ATCC, Rockville, MD), and Korean Collection for Type Cultures (KCTC, Daejeon, Korea), Korea Culture Center of Microorganisms (KCCM, Seoul, Korea) (Table 1). All clinical isolates were provided from Yonsei University College of Medicine (Seoul, Korea).

2.2. Genomic DNA isolation from reference bacteria and clinical specimens

Cells of reference bacteria were suspended in 180 μl of lysozyme buffer (20 mM Tris–Cl, pH 8.0, 2 mM EDTA, 1.2% Triton, 20 mg/ml lysozyme) and were incubated at 37 °C for 30 min. DNA was isolated using the DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA from clinical specimen was extracted from 1 ml aliquot of the overnight culture. The cell pellet obtained by centrifugation at 10,000×*g* for 5 min was resuspended in 200 μl of phosphate-buffered saline (PBS) solution. DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's instructions.

Table 1
Bacterial strains used in this study

Species	Source
Acinetobacter baumannii	KCTC 2771
Pseudomonas aeruginosa	KCTC 1636
Escherichia coli	ATCC 25922
Klebsiella pneumoniae	ATCC 700603
Streptococcus pneumoniae	KCCM 40410
Vibrio vulnficus	KCTC 2962
Aeromonas hydrophila	KCCM 32586
Klebsiella oxytoca	ATCC 43863
Staphylococcus epidermidis	KCTC 1917
Burkholderia cepacia	ATCC 25416
Salmonella typhimurium	KCCM 40253
Salmonella enteritidis	KCCM 12021
Proteus mirabilis	KCCM 11381
Listeria monocytogenes	ATCC 700603
Enterococcus faecium	ATCC 19434
Staphylococcus aureus	KCTC 1621
Neisseria meningitidis	ATCC 13100
Cardiobacterium hominis	ATCC 14900
Chryseobacterium meningosepticum	ATCC 13253
Ochrobactrum anthropi	ATCC 49188
Comamonas acidovorans	ATCC 9355
Peptostreptococcus prevotii	KCTC 3319
Anaerobiospirillum succiniproducens	ATCC 29305
Bacteroides fragilis	ATCC 25285
Porphylomonas gingivalis	ATCC 33277
Stomatococcus mucilaginosus	ATCC 17931

ATCC, American Type Culture Collection; KCTC, Korean Collection for Type Cultures; KCCM, Korea Culture Center of Microorganisms.

2.3. Sequencing of 23S rDNA and 16S–23S rDNA ISR of *A. baumannii*

The 23S rDNA and the 16S–23S rDNA ISR of *A. baumannii* were amplified by PCR with the universal primers, 1585Fw and 2960R (Table 2). The PCR product was cloned into the NotI site of pGEM Easy T Vector system (Promega Co. Madison, WI). The cloned PCR product was sequenced with DNA Analyzer (ABI Prism 3700; Perkin–Elmer, Forster City, CA).

2.4. Oligonucleotide microarray fabrication

The species-specific probes were first designed by BLAST and multiple alignment of 23S rDNA and 16S–23S rDNA ISR sequences (see Section 3). Multiple alignment was carried out using Vector NTI version 6.0 (InforMax. Inc., Bethesda, MD).

Table 2
Primers used in this study

Primer name	Sequence (5′-3′)	Reference
1585Fw	TTGTACACACCGCCCGTC	This work
2960R	GCTTAGATGCTTTCAGCG	This work
23BR	Cy3-TTCGCCTTTCCCTCACGGTACT	[12]
23Fw	AGTACCGTGAGGGAAAGGCGAA	[12]
MS37R	Cy3-TGGCTGCTTCTAAGCCAACATCCT	[14]
MS37Fw	AGGATGTTGGCTTAGAAGCAGCCA	[14]
MS38R	Cy3-CCCGACAAGGAATTTCGCTACCTT	[14]