Research Article

A New Microarray System to Detect *Streptococcus pneumoniae* Serotypes

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Streptococcus pneumoniae, one of the most common gram-positive pathogens to colonize the human upper respiratory tract, is responsible for many severe infections, including meningitis and bacteremia. A 23-valent pneumococcal vaccine is available to protect against the 23 *S. pneumoniae* serotypes responsible for 90% of reported bacteremic infections. Unfortunately, current *S. pneumoniae* serotype testing requires a large panel of expensive antisera, assay results may be subjective, and serotype cross-reactions are common. For this study, we designed an oligonucleotide-based DNA microarray to identify glycosyltransferase gene sequences specific to each vaccine-related serotype. Out of 56 isolates representing different serotypes, only one isolate, representing serotype 23A, was not detected correctly as it could not be distinguished from serotype 23F. Our data suggest that the microarray provides a more cost-effective and reliable way of monitoring pneumococcal capsular types.

1. Introduction

Streptococcus pneumoniae is an important cause of bacteremia, community-acquired bacterial pneumonia, and meningitis, especially among young children and older adults [1–3]. Capsular polysaccharide is the primary *S. pneumoniae* virulence factor and encapsulated pneumococci are responsible for more diseases than unencapsulated strains [4]. After comparing the differences in capsular polysaccharides composition, *S. pneumoniae* can be divided into more than 90 serotypes [5] and the 23 serotypes responsible for 90% of disease cases [6] are represented in a 23-valent pneumococcal vaccine. Pneumococcal serogroup and serotype identification is currently performed by using large panels of expensive antisera by various methods, including the capsular swelling (Quellung) reaction, latex agglutination, and coagglutination. Cross-reactions between serotypes and discrepancies between methods can occur and some strains are nonserotypable. On the other hand,

molecular typing has the potential to improve discrimination and provide additional information. *S. pneumoniae* capsule production is predominantly controlled by capsular polysaccharide synthesis (*cps*) gene clusters [7, 8], which are responsible for each serotype-specific polysaccharide. The Sanger Institute has sequenced the *cps* gene clusters of 90 *S. pneumoniae* serotypes and predicted the general function of 1,973 of the 1,999 gene products [9, 10]. *S. pneumoniae* capsular polysaccharides represent a diverse group of polymers with distinct sugar compositions and linkages [10]. The key enzymes to link each serotype-specific sugar component are glycosyltransferases (GTs) [11], which transfer the sugar moiety from an activated nucleotide sugar to an acceptor to generate a serotype-specific capsular polysaccharide. After discovering that *S. pneumoniae* GT genes are highly variable and contain serotype- or serogroup-specific regions, we used GT sequences as probes in an oligonucleotide-based microarray to identify 23-valent pneumococcal vaccine and closely related *S. pneumoniae* serotypes. Our data suggest that

the microarray provides a more cost-effective and reliable way of monitoring serotype distribution.

2. Materials and Methods

2.1. Bacterial Strains, Growth Conditions, Immunological Serotyping, and Genomic DNA Extraction. *S. pneumoniae* strains representing various serotypes were obtained from the American Type Culture Collection, the Statens Serum Institute, and clinical isolates (Table 1). Each strain was cultivated on brain-heart infusion broth (Eiken, Tokyo, Japan) supplemented with 0.3% yeast extract (Becton Dickinson, Boston, MA) (BHI-Y) for 24 h at 37°C in 5% CO_2. Conventional serotyping was performed for clinical isolates obtained in Japan by slide agglutination (Denka Seiken, Tokyo, Japan) or quellung reaction (Statens Serum Institute, Copenhagen, Denmark).

Genomic DNA was extracted using a Wizard Genomic DNA purification kit (Promega, Madison, WI).

2.2. DNA Array Preparation. Oligonucleotide probes were synthesized and spotted on a glass slide at Nihon Gaishi (Nagoya, Japan). The slide was stirred in a beaker filled with $2 \times$ SSC/0.2% SDS for 15 min, transferred to a second beaker filled with $2 \times$ SSC/0.2% SDS to incubate for 5 min at 95°C, rinsed three times with dH_2O, and centrifuged at 900 rpm for 3 min at 25°C in a horizontal microtiter plate rotor before being covered with a plastic seal.

2.3. Chromosomal DNA Labeling. 500 ng of genomic DNA was suspended in 21 μL dH_2O and 20 μL of $2.5 \times$ Random Primer Solution (Invitrogen, Carlsbad, CA), heated to 95°C for 5 min, and chilled on ice for 3 min. The DNA was labelled in a reaction including 5 μL of 10X dCTP Nucleotide Mix (Invitrogen, Carlsbad, CA), 5 μL Cy3 or Cy5-dCTP (GE Healthcare, Buckinghamshire, UK), and 1 μL of Exo-Klenow Fragment (Invitrogen, Carlsbad, CA). After a 2-hour incubation at 37°C, 5 μL of sodium acetate, 125 μL of ethanol and 1 μL of glycogen was added to 25 μL of Cy3 and Cy5 labeled DNA, which was purified previously by QIAprep Spin Miniprep Kit (250) (Qiagen, Tokyo, Japan). Following a 30-minute incubation at −80°C in the dark, the probe mixture was centrifuged for 30 min at 14,000 rpm at 4°C. The supernatant was removed and the probe was air-dried for 5 min in the dark. The probe mixture was diluted in 70 μL of the hybridization buffer (25% formamide, 0.1% SDS, 6 $\times$ SSPE), incubated for 30 min at room temperature in the dark, heated for 8 min at 75°C, and incubated for 30 min at 42°C.

2.4. Probe Hybridization and Microarray Signal Detection. Prewarmed probe mixture was applied to the prepared microarray slide, placed in a hybridization chamber and incubated for 20 h at 42°C. After hybridization, the plastic seal was removed and the slide was washed with 1 $\times$ SSC/0.1% SDS solution for 3 min, 0.05 $\times$ SSC for 3 min, and 95% ethanol for 90 s at room temperature. The washed microarray slide was dried by centrifugation and scanned

TABLE 1: Test strains.

Serotype	Strain designation	Serotype	Strain designation
1	ATCC6301[a]	**14**	D59[b]
2	ATCC6302[a]	15F	ATCC6315[a]
3	D36[b]	15A	ATCC6330[a]
4	JHK27[b]	**15B**	ATCC10354[a]
5	ATCC6305[a]	15C	SSI15C/2[c]
6A	MSC1943[b]	**17F**	ATCC6317[a]
6B	MSC1047[b]	17A	SSI17A/2[c]
7F	ATCC10351[a]	18F	ATCC6318[a]
7A	ATCC6307[a]	18A	ATCC10344[a]
7B	ATCC10348[a]	18B	ATCC10355[a]
7C	ATCC10350[a]	**18C**	ATCC10356[a]
8	ATCC6308[a]	**19F**	D33[b]
9A	ATCC8333[a]	**19A**	D4[b]
9V	KD10-11[b]	19B	ATCC10358[a]
9L	ATCC10349[a]	19C	ATCC10359[a]
9N	KD01-26[b]	**20**	ATCC6320[a]
10F	ATCC6310[a]	**22F**	KD01-23[b]
10A	ATCC8334[a]	22A	ATCC10363[a]
10B	SSI10B/2[c]	**23F**	KD11-15[b]
10C	SSI10C/2[c]	23A	KD12-06[b]
11F	ATCC6311[a]	23B	ATCC10364[a]
11A	SSI11A/2[c]	**33F**	ATCC10370[a]
11B	SSI11B/2[c]	33A	ATCC8340[a]
11C	ATCC10353[a]	33B	ATCC10342[a]
11D	SSI11D/1[c]	33C	ATCC8339[a]
12F	ATCC6312[a]	33D	SSI33D/2[c]
12A	SSI12A/5[c]	44	SSI44/3[c]
12B	SSI12B/1[c]	46	SSI46/2[c]

Explanatory notes: Serotypes represented in bold letter are those included in 23-valent pneumococcal vaccine.
[a]American Type Culture Collection.
[b]Clinical isolate obtained from Japan.
[c]Statens Serum Institute.

using the DNA Microarray Scanner (Agilent, Santa Clara, CA).

2.5. Data Analysis. The signal and background intensities of each spot were quantified using GenePix Pro 6.0 software and the average was calculated with Microsoft Excel software.

3. Results

3.1. Target Gene Selection and Microarray Construction. In this study, we designed a DNA microarray to identify the 23 *S. pneumoniae* serotypes included in the 23-valent pneumococcal vaccine, using GT genes in *cps* locus. We compared the GT sequences of the 23-valent vaccine serotypes with other *S. pneumoniae* serotypes and found that these 23 serotypes were indistinguishable from 14 nonvaccine serotypes. Therefore, 37 serotypes, 23-valent vaccine serotypes and 14 closely related serotypes, were divided into 23 groups and each group had one to six GT genes in their *cps* locus

TABLE 2: Twenty-three groups distinguished in this study and targeted glycosyltransferase genes.

Group name	Targeted GT genes in cps locus (probe number[a])					
1	**wchB** (1, 2, 3)	**wchD** (4, 5, 6)				
2	**wchF** (7, 8, 9)	**wchG** (10, 11, 12)	**wchH** (13, 14, 15)	**wchI** (16, 17, 18)		
3	**wchE** (19, 20, 21)					
4	**wciJ** (22, 23, 24)	**wciK** (25, 26, 27)	**wciL** (28, 29, 30)			
5	**wciJ** (31, 23, 24)	**whaC** (32, 33, 34)	**whaD** (35, 36, 37)			
6A/6B	**wciN** (38, 39, 40)	**wciP** (41, 42, 43)				
7F/7A	**wchF** (44, 45, 46)	**wcwA** (47, 48, 49)	**wcwF** (50, 51, 52)	**wcwG** (53, 54, 55)	**wcwH** (56, 57, 58)	
8	**wciR** (59, 60, 61)	**wciR** (62, 63, 64)	**wciS** (65, 66, 67)	**wciT** (68, 69, 70)		
9A/9V	**wchO** (71, 72, 73, 74)	**wcjA** (75, 76, 77)	**wcjB** (78, 84, 85)	**WcjC** (81, 82, 83)		
9L/9N	**wchO** (71, 72, 73, 74)	**wcjA** (75, 76, 77)	**wcjB** (78, 79, 80)	**wcjC** (81, 82, 83)		
10A	**wciB** (86, 87, 8)	**wcrC** (89, 90, 91)	**wcrD** (92, 93, 94)	**wciF** (95, 96, 97)	**wcrG** (98, 99, 100)	
11A/11D	**wchK** (101, 102, 103)	**wcyK** (104, 105, 106)	**wcrL** (107, 108, 109)			
12F/12A/ 12B/44/46	**wciJ** (110, 111, 112)	**wcxB** (113, 114, 115)	**wcxD** (116, 117, 118)	**wcxE** (119, 120, 121)	**wcxF** (122, 123, 124)	
14	**wchK** (125, 126, 127)	**wchL** (128, 129, 130, 131)	**wchM** (132, 133, 1334)	**wchN** (135, 136, 137)		
15B/15C	**wchK** (138, 139, 125)	**wchL** (128, 140, 141, 131)	**wchM** (142, 143)	**wchN** (135)		
17F	**wchF** (144, 145, 146)	**abp1** (147, 148, 149)	**wciP** (150, 151, 152)	**wcrV** (153, 154, 155)		
18B/18C	**wchF** (156, 157)	**wciU** (158, 159, 160)	**wciV** (161, 162, 163)	**wciW** (164, 165, 166)		
19F	**wchO** (167, 72, 168, 169)	**wchQ** (171, 172, 173)				
19A	**wchO** (71, 170, 73, 74)	**wchQ** (171, 172, 173)				
20	**wciB** (174, 175, 176)	**whaJ** (177, 178, 179)	**wciL** (180, 181, 182)	**wcwK** (183, 184, 185)	**wciD** (186, 187, 188)	**whaF** (189, 190, 191)
22F/22A	**wchF** (7, 8, 192, 193)	**wcwA** (48, 49, 194)	**wcwV** (195, 196, 197)	**whaB** (198, 199, 200)		
23F	**wchF** (144, 156, 145, 193, 201)	**wchV** (202, 203, 204)	**wchW** (205, 206, 207)			
33F/33A/ 37	**wciB** (208, 209, 210)	**wciC** (211, 212, 213)	**wciD** (214, 215, 216)	**wciE** (217, 218, 219)	**wciF** (220, 221, 222)	

Explanatory notes: [a]Probes containing 60-bp oligonucleotides were designed and named as 1, 2, 3 etc from Group 1. The name of each GT gene (*wchB* etc) was derived from the Sanger Institute.

(Table 2). The 60-bp oligonucleotide probes contained the variable middle region of each open reading frame and were designed from published sequences at the Sanger Institute (http://www.sanger.ac.uk/Projects/S_pneumoniae/CPS/) and Genbank websites. In most cases, the designed probes were gene specific, although some probes included sequences from more than one gene. Each serotype group was identified using 3 to 18 probes (Table 2) and a total of 222 probes were designed to target 23 groups (Table 3). 26 positive control probes were designed to hybridize *S. pneumoniae* housekeeping genes and 16S rDNA. In addition, 26 negative control probes were designed to detect housekeeping genes of other bacterial respiratory pathogens, including *Klebsiella pneumoniae, Staphylococcus aureus, Legionella pneumophila, Chlamydophila pneumoniae, Mycoplasma pneumoniae, Pseudomonas aeruginosa,* and *Streptococcus pyogenes*. A schematic

Table 3: Oligonucleotide probes used in this study.

Spot identifier	Targeted GT gene	Specificity	Probe sequence (5′–3′)
1	*wchB*	Serotype1	ATAAGATTATTGAGAAAATATAGACCGGATGTAGTCTTGACATATACC-GTGAAACCAAAT
2	*wchB*	Serotype1	TTTATTGGTAGGATATTAAAAGAAAAAGGTATAGATACTTATCTGGCTGCT-GCCCAAATT
3	*wchB*	Serotype1	GAAAATGAAAAACGAAAAGAGATGGGACTTCAAGGGAGAATGTATATA-GAGCAATATTTT
4	*wchD*	Serotype1	TTATTGAAGGAATGATTGATAGCGACTTAATAGTTGTTCGTATTCCGTCTA-TAATTGGAT
5	*wchD*	Serotype1	GCCATAGATTTGTATTGGAAGCAATGAAGAGATTAGAAATACAAGGTA-TTTTGTTGGATT
6	*wchD*	Serotype1	AGCGATTGCGGGATCTATTATAGATTTTATTAGTATGGATAAGGAAAA-GATGGTGATAAA
7	*wchF*	Serotype2, 21, 22F, 22A,23B, 32F, 32A	TTTGTTGAGAAATTAACAGAATATCAAAAGATGGTAACATCCAATACTAT-GTTGCCTGC
8	*wchF*	Serotype2, 21, 22F, 22A, 27, 32F, 32A	CTAAAAAAGACTTTGTTCTCATTACAAATGTGGAACAGAATAAGTTTTACG-ATCAGTTGC
9	*wchF*	Serotype2	TTATTGAAGCAGTGGAGCAATTTGATGAGAACGCCATTTCTGAACTAG-ATAAAAAATCTA
10	*wchG*	Serotype2	GCAATACCAAGAAAAATACCCTAAAAAAATTAAGGTTATCACAGATTCCTC-TGTTATAGG
11	*wchG*	Serotype2	TAGAAGTTTAAACAATCTGTTAGATTTGAATAGTAATGCAGTAGCTATGCA-TGATTGGTG
12	*wchG*	Serotype2	TTATCAGAATTCTCTAAGTAATGAGGAGACAGATATTATTCGTGAATTTAT-CAGCATTCC
13	*wchH*	Serotype2	TAGAAACCAGACAATTTTTTATCGGATAAAAGCTTCTTTGGGGAATACTCT-AAAAAACG
14	*wchH*	Serotype2	CGTATTCCAGAAAAGTTACCTGATACCTATAATGTGTTGATTAATCCTGAA-AGAGAAAAA
15	*wchH*	Serotype2	CTTTGTTGGAACTCTCAAATGGTCAGAATACTATAGTTGTAGAAGAGTTAT-CAGAAATAT
16	*wchI*	Serotype2	CATTTTACCAGAACATGGAAATGTGGAAGATGAGCTTGTAAACAAAGG-AATTAAATACTA
17	*wchI*	Serotype2	TGATTTAGTTAGAGCGATAGCTAATCTTCCTGAGAGATATAAACAGATGTT-TAAAGTTGA
18	*wchI*	Serotype2	TACAAAAGAGATAATTTCTACAGGAGAAACAGGATATCTGTATGAACC-AGGAGATTATAT
19	*wchE*	Serotype3	TATAAGTCCTACAGTTGTAGTGTAAGTGATGAGAAGTTATTTAGTTCTGTA-ATTATCCCT
20	*wchE*	Serotype3	ACTTTAAAAAAAGGCTATAAAACTGTTATGCAGGATACTTCTGTTGTGTAT-ACAGATGCT
21	*wchE*	Serotype3	ACTGCAATTGTTTATACAGCTTCATGGTGGGAAATTATTTTATATGTTCTT-TTGGGAATG
22	*wciJ*	Serotype4, 45	GATTGTTGAATTATTTTAGCTTTGCAATTAGTTCTACTTTAGGAGTTTTAT-TGGGGAGGT
23	*wciJ*	Serotype4, 5, 45	GCCACAATATGCAGAAGATCTTTTTATCCCTGATGAATCTATAGTTAATAA-AGAAAGTGT
24	*wciJ*	Serotype4, 5, 45	CCTTCTATAAAAAATCAGATGCTATGTTAGTTTCTTTAATAGGAGACTCGA-TAGTTTCTC
25	*wciK*	Serotype4	GGTTCAGAAACAATTGGTGAAAAATTCTTTAATGAATATCGTTTCTTCAGA-CGGCTATAA
26	*wciK*	Serotype4	TCGATTTCAGTTGAATTTTATAGGTACTAATGCAGGAGAATTAAGGGAATT-TTGTCAAGA

TABLE 3: Continued.

Spot identifier	Targeted GT gene	Specificity	Probe sequence (5′–3′)
27	*wciK*	Serotype4	GTGAAGATACTTATATGGAAAAAGTGTCAATAGAGAATGGTTTTGGTT-TCGTTTTACCTA
28	*wciL*	Serotype4	AAAAGCCTCTACATCAGTTTCTCTCTCTTGCTAGAATAATAAAGAAAGGAG-ATTATGATA
29	*wciL*	Serotype4	AGAACTCATTTTAATCAAACCAAATGTTATTTTACTCCTAGTTGGTAATGG-TGAGGATGA
30	*wciL*	Serotype4	AAAAACATTAGTTACTTACCTATCAACGAAGAGTCTGTGTTGCTATGGAAA-GATAAAGTA
31	*wciJ*	Serotype5	TTACATAGGATATTAAATTATTTTAGTTTTGCTATCAGTTCCTCGATAGGG-GTTCTACTG
32	*whaC*	Serotype5	TTTCTGACTCTCACAAGTATGATGGATTGGTATTACCAAAGAAAAATACAG-TTCGCAATT
33	*whaC*	Serotype5	TATATCCCGAACCTCAACTTTTGAACCTTTTAACGAGAAATATCATATCCG-TCAGATTAT
34	*whaC*	Serotype5	GAAGACTAAACTTCAGCGTGAATTGAAACTAGAAGAAGCACGCTATAA-AGGAAATAGATT
35	*whaD*	Serotype5	AAGACGGCAGTACGCTATTTCTGTTGATGGTATAATAAATCATAGTAATAT-CTCACTTAA
36	*whaD*	Serotype5	GTTTTCACAAGATATAGTATTCGAAAATCTGAGAAAAATCTGCTTTTTGTG-GGACAGTTT
37	*whaD*	Serotype5	TCTAATATACATATAATTCCTTTTCTAGAAAAAACTGATATCCTAGAGTTG-ATGCGGGTG
38	*wciN*	Serotype6A, 6B, 33D	AATAGATTATCAAAACAATTTGCGCAGAGAGAAATTAATTGGATAGAG-AACGTTGAGATC
39	*wciN*	Serotype6A, 6B, 33D	TTACAAGGAGATTTAGGGGTTTTAAATGCAGTTTTATATAACTCATTTGGT-GTACTTCCT
40	*wciN*	Serotype6A, 6B	GCAAGAAGGCAGTAATGTTGCACATATAGACCAATTTAAAAAATACTA-TGAAGGTAGTTA
41	*wciP*	Serotype6A, 6B	GGACACTTTTTATTAGGGATGATGGATCAAAAGATAAAACAATAGAAG-TAATACAGAGGT
42	*wciP*	Serotype6A, 6B	CAGGTTTTAATCATGCATTGCTAGAGATGGTTCCTTCAGTTGATATTGATA-AAGATTATT
43	*wciP*	Serotype6A, 6B	CTTACACATGCTGGGGTATATAATCAAACTCTTTATATGCTAAAAAAAGCT-TCTGGAAAA
44	*wchF*	Serotype7F, 7A	ATACAATACTATGTTGCTTGTATGCGTGAAAATTCAGCTAAATCTGGCATC-ATGGATGAT
45	*wchF*	Serotype7F, 7A	AAAAATATATCCAAGAGGATTATAAGCAGTACCAACCAAAGACCACCT-ATATTGCCTATG
46	*wchF*	Serotype7F, 7A, 23B	TTGTTACAGGATACTGGTTTTGATAAAGATCCTAGGGTTAAATTTGTT-GGGACTGTCTAT
47	*wcwA*	Serotype7F, 7A	AAGTGCATCTTTCCAACGTCAAAAAGAATTCTTTTCGTTGGAAAGTTATAT-TCGGAATTT
48	*wcwA*	Serotype7F, 7A, 21, 22F, 22A	GTGTGTTGGATTATCCGATTTTACGTAGAAAATACTTTAATCCTAAGGGGA-TTTTAGAAT
49	*wcwA*	Serotype7F, 7A, 21, 22F, 22A	CTCACAAATCAAACGAATTGACTATTATGAGCATACGACTGAGCTTTATAA-TATGTTTGA
50	*wcwF*	Serotype7F, 7A	AAATATGAAGTTATTCTAGTAAATGATGGCAGTACAGATGCTTCACCCAAT-ATTTGTGAA
51	*wcwF*	Serotype7F, 7A	TATTTTATTGGGAATGATGCGGCTATTACCAAACAGTGGTCTGAAAAA-AAAATTAGTGAT
52	*wcwF*	Serotype7F, 7A	ATGAAATTGTATGAAGAAAATCAGGAAGACACTCAACTTTTTAGGTTGATA-CTTGCAGAA

TABLE 3: Continued.

Spot identifier	Targeted GT gene	Specificity	Probe sequence (5′–3′)
53	*wcwG*	Serotype7F, 7A	AAAACGATTACCCGGATTTTTATTCCATAATTGGTGGTTAGAAGAATGGTC-TAGAAAATT
54	*wcwG*	Serotype7F, 7A	GGTGCAGATAAAGGAAGATTGCCAAAATTAAAAAGCTTAGCTAAGCAG-ATAGTTTTAAAT
55	*wcwG*	Serotype7F, 7A	ATAAAAAGGGACAGGATGGTCTAACCCTTAGAGCAATGGAATCCATTT-TTTATAAAAAAA
56	*wcwH*	Serotype7F, 7A	GGAACAGAGTTACTAAGAATTGTAAAATCAAATCAATTGTAGGCAATA-TACGTGGCAAAA
57	*wcwH*	Serotype7F, 7A	ATTTGCTAAATCCTATAGAGAAACGAAACCCATTTCATCTAGGTATGTTAT-ATCATGAAG
58	*wcwH*	Serotype7F, 7A	TATTTGAATATGCAATTGATGGCGAGAATGCACTTTTATCTCCGATAAAAG-ATAGTGTTA
59	*wciO*	Serotype8	AACTAATGAAGCTTGAACCGATTATGAGACAACAAGACAGCTATTTAA-TCACAGAATATA
60	*wciO*	Serotype8	AAATCACTTTATATACTGTTAAGAATACGCCCAAAAGTAGTTATCTGTACA-GGTGTTCTT
61	*wciO*	Serotype8	TTTATATTGAATCTTTTGCAAAAGTGACCACTCCTACTTTAACAGGTAGAA-TACTATACC
62	*wciR*	Serotype8	AAAATAGATCAACTTATTGAATTAGAAGTGATAAAGGAAGAGGTGTTT-GCTCAGATTGGA
63	*wciR*	Serotype8	TAAAGCTTTGAAATTAAGAAAAAAAATTATAGCGGTTCCACGATTAGA-GCAGTTTGGAGA
64	*wciR*	Serotype8	ATGCTTTGATATAGAGCAGTTAGGAACTGTTTATCAAAAAGCTCAGACTTT-TACAACAAA
65	*wciS*	Serotype8	TTTATTGATGGCTCTCTTGTAACACGGTTAACCTATTCTAGTTATGCTCTT-CTTAAATTT
66	*wciS*	Serotype8	AACAACTTTCTTTTTTTAGGAAGGATGGGCAAAAGAAAAGGAGCCTAT-GATTTAATAGAT
67	*wciS*	Serotype8	AGGATAATGGCTGGTTAATTCAACCGGGTGATATTTCTCAGTTATCTAATA-TTATTTTAG
68	*wciT*	Serotype8	ATGGAATGAGGATAATTTTGATTTATCAGATTCACAATTTGCGAAGTCTGC-ATATGAATC
69	*wciT*	Serotype8	GGTGCAATATTATGAGCAAGCAAGTTTTGATATCAATCATTTGGTAACTGT-CAATACAAT
70	*wciT*	Serotype8	AATAATTGATGGATTAGCAATTTATCCAGATGATTACTTTTGTGGTTATGA-TCAGGAGGT
71	*wchO*	Serotype9A, 9V, 9L, 9N, 19A	ATTAACGATGAAAGAAACAGTGGATGCTGTTGAACAGTATGTTTTAAA-GAAGCATCCTTT
72	*wchO*	Serotype9A, 9V, 9L, 9N, 19F, 19B, 19C	TTTTGATGTATTATCAGGACACATTAAACGAGCTCCATTATGGATGCAAAA-ATTGAATCT
73	*wchO*	Serotype9A, 9V, 9L, 9N, 19A	GAAAGAATATATTATCCAATCATTCATGGATAATGGAATTAATGCTGTGTT-TATGGGGGT
74	*wchO*	Serotype9A, 9V, 9L, 9N, 19A	GAGTAGCGGGTATTGATTTGATGCAATGTCTTTTAGAGTTGTCAAATA-AAAAAGGATATT
75	*wcjA*	Serotype9A, 9V, 9L, 9N	AACAGGTGGACTATGGGAGAGCAAACTTTTATCAAAAGGAGTTCAACA-TCATAAAATTTT
76	*wcjA*	Serotype9A, 9V, 9L, 9N	TTAAAAAAAGCGTATTGTGTAGCTGTGGGTAAAGCGGTTAATGATAAT-TTGAAACATGAT
77	*wcjA*	Serotype9A, 9V, 9L, 9N	GTTGTTGAATGTATCAATAGTTTTGATTACTTAGTGTCATCATCTTTATAT-GAGGGGTTG

TABLE 3: Continued.

Spot identifier	Targeted GT gene	Specificity	Probe sequence (5′–3′)
78	*wcjB*	Serotype9A, 9V, 9L, 9N	GAAAAGGCTAATTTAGAAAATGAACTAATTGTTTCGTTTACAACAATTCCA-AGCCGTCTT
79	*wcjB*	Serotype9L, 9N	AGTTGTTCTAGTTGATGACGATATCATTTATCCTCGAAATACTATAAAGAA-ACTGATTGC
80	*wcjB*	Serotype9L, 9N	CAATCCTGAGGAGAGTTTGGTATATTTGAATACCGTATATGATAACAA-CAATGATAAATG
81	*wcjC*	Serotype9A, 9V, 9L, 9N	AAATTTCTAGCTGAACAACTTGTAAAAGAAGGACATGAGGTATTTGCA-TACTCTGATGAT
82	*wcjC*	Serotype9A, 9V, 9L, 9N	TTATCAATAAAGGATTTATTAACCCATCTTCTCAAAAATGTATGGCCATTG-AAAATGCTG
83	*wcjC*	Serotype9A, 9V, 9L, 9N	CGAAAGTGATCCTAGAATACAATATTTAGGCTTTCAAGATACAAAAAACCT-CTATGAAAC
84	*wcjB*	Serotype9A, 9V	ATTGTAATTTTGGTTGATGATGACACTGTCTATTCATCGAATACCATCGAA-AAGTTAGTT
85	*wcjB*	Serotype9A, 9V	ACCCTGAAGAGAGTTTGGTGTATCTGAATGCTATATATGATAATAATAATG-ATAGGTGTA
86	*wciB*	Serotype10F, 10A, 10B, 10C, 47A	ATCAAGGTAATCATATCTCACACCTCAATCCTTATTATTGTGAATTGACAG-GATTATACT
87	*wciB*	Serotype10F, 10A, 10B, 10C, 31, 47A	TTTAGATGTAACGCGAGAAATTATAAAAGAGGTTTCGCCAGAATATTT-AGCAACATTTGA
88	*wciB*	Serotype10F, 10A, 10B, 10C, 47A	TGAATTTATTTGAGAAGGGCAAATCCTTCTTGAAAGCCAAGTATTTCG-GAAAAAAATATG
89	*wcrC*	Serotype10A, 10C, 34, 35F, 43, 47F, 47A	GTTGCTGTATCTTTGGCAAACGAACTTACAAAAAAGTATGAAGTTCATTTG-ATTGGAATT
90	*wcrC*	Serotype10A, 10C	TAACTGTTGGTCGTTTTGATTATCAAAAAGGATATGATTATCTTATCCAAG-TCGCGAAAA
91	*wcrC*	Serotype10A, 10C	TGGTTATCTGATAGATTGTTATGATACCGATAAGATGAGTGAGAAATT-GCTTGAATTGAT
92	*wcrD*	Serotype10A, 10B	GGATATGGTTCTTACGGATTTTACAGAACAACATGTTTATAACAATACTAC-TGTTCGAAA
93	*wcrD*	Serotype10A, 10B	ACCTATAGAACATCTATCCTAGTCGACAATAGAATTCGTTTAAGTGAAAAG-ACGTTTTAT
94	*wcrD*	Serotype10A, 10B	TATCAGAAGAATTATACAGACAAATTGAGCAGAGTTCTTATGAGTATATCC-CTACGAAAA
95	*wciF*	Serotype10A, 10B	AAGCATCATCAGATTGGATTTTCTTTCTAGATCCAGATGATTATTTGGAAG-ATTATACTC
96	*wciF*	Serotype10A, 10B	GGATAAAATTGTGATTAGTCCACTTGAAACATATAACTATTACCGTAGAGA-AGGTAGTAT
97	*wciF*	Serotype10A, 10B	AGGCTGACTCTGGTTTAACAGATTTTTCGAAAGATCGAAACCTATTAA-AAGTTGATTTTA
98	*wcrG*	Serotype10A, 10B	CTCTGTTGGATTATAAGGAACATGATATTTTTATTATTGTAGGCAGCAAAG-TTAATGTGG
99	*wcrG*	Serotype10A, 10B	GCTAGAAATATTCAGAACAAATATGTTCGTAAATTTGTAGCATATTACCGT-AAGCTAGAG
100	*wcrG*	Serotype10A, 10B	GCATCTAACTGGGTATCTATTAATCAGGATTTAGTTAGAATAATACTAGAA-GAAGAGAAA
101	*wchK*	Serotype11A, 11B, 11C, 11D, 14, 15F, 15A	GATAGATTAAAAGGTGAGGGATTTATTCAGGATGATGTTTTTATTCAGACT-GGTTTTTCA

TABLE 3: Continued.

Spot identifier	Targeted GT gene	Specificity	Probe sequence (5′–3′)
102	*wchK*	Serotype11A, 11D	TTCTTATGATGAGATGAATCGCTATATAGATGAAGCAAATATTATCATTAC-ACATGGCGG
103	*wchK*	Serotype11F, 11A, 11D	CGAAGGGTATGAATTATCTCTGATTAATGATATAAGCGAATTGCAGTA-TAGTTTAAAGCA
104	*wcyK*	Serotype11F, 11A, 11D	CATCATAAATTAGATAAACTACTACGACCGATATTTTATCGAGTTTATACT-CAGGCATGT
105	*wcyK*	Serotype11F, 11A, 11D	CTAGGTCATGTTGGACGTTTTAATACTCAAAAAAATCAATGTTTTCTAGTG-TCTCTAATG
106	*wcyK*	Serotype11F, 11A, 11D	GGTCAATTTGATGATATGAAATCTTTTGTGTCATCAATGGATATAATGTTG-CTTCCAAGT
107	*wcrL*	Serotype11F, 11A, 11D	TGTATTGAAAATCAAAATCAATTTGTGCAGGATGCATATAGAGATAAA-GCATGGGCTTTT
108	*wcrL*	Serotype11F, 11A, 11D	GTTATGTCCTGAATTAAATACACCTGTATTTAAACGTCTTGGTTATACTTA-TTCTGACTG
109	*wcrL*	Serotype11F, 11A, 11D	CAGATAAAACATTCTCTATTCATCATTATAGTGCTTCTTGGACTTCCTTAA-GAAATCAGA
110	*wciJ*	Serotype12F, 12A, 12B, 44, 46	GGAAATATATGCTGATTATCGTAAGAGAAAAAAAGAAGAGAGACTAT-AAATGGTGTTGC
111	*wciJ*	Serotype12F, 12A, 12B, 44, 46	ACTTAACTTTTGCTGGAAATATTGGAAAAGCTCAGAATTTAGAGACTATTT-TGAAAGCAG
112	*wciJ*	Serotype12F, 12A, 12B, 44, 46	AATGTTGATCAGTTAGTGAGAAATATTCGTAAGTTCTGTTTGCTTTCTGTA-GAGGAAAGA
113	*wcxB*	Serotype12F, 12A, 12B, 44, 46	ATGTTCCGAAACAATTTCAACAGTATGCAGTGAAAATTGGTACAAAGT-CTGATATTCGTT
114	*wcxB*	Serotype12F, 12A, 12B, 44, 46	AAAAGAATATCCAGTGAAAGTAATTCATAATGGTATTGATACTACTGTCTT-TCAACCGAG
115	*wcxB*	Serotype12F, 12A, 12B, 44, 46	TAGAAAGTGCTAAACTTTATGGTCTCGTTTGTCAGGATAGAAACGTAG-CTTCTATTTTAT
116	*wcxD*	Serotype12F, 12A, 12B, 44, 46	GAAGAAGAATTTTTTTAAAGTTAGTGGAGCTTTGCGAAAAGTGTTGAA-AAAACAGCAGTT
117	*wcxD*	Serotype12F, 12A, 12B, 44, 46	TGTCAGCTCTCTTCTAAAAAAATTATCAGTGTTGGATCTTTAGTACGACAA-AAAGGTTTT
118	*wcxD*	Serotype12F, 12A, 12B, 44, 46	GATAGAGAAAAATTAGAGGAGAAAGTCAGGGAATACCAATTAGAAGGC-TTTATAAATTTG
119	*wcxE*	Serotype12F, 12A, 12B, 44, 46	ATAAAATCCCCGATAATCTTACCCAATTTTTTGGACGAGAAAATATAGAAG-AGAGAGATA
120	*wcxE*	Serotype12F, 12A, 12B, 44, 46	TATAAAACCTTGATTACTCCCATTTTGATAAAAGAACAGATACCAATTATT-CGGACGCAA
121	*wcxE*	Serotype12F, 12A, 12B, 44, 46	AGGTAGCAGATTTTGCTTTATTTCCTAAACAATGTAGTTTAAGTTTTTATG-ATGCACAGG
122	*wcxF*	Serotype12F, 12A, 12B, 44, 46	AAGTTACAATGAGAAATATAATCATGATGAAATTACGGTCGTTAGTTGTGA-CCATAAGGA

TABLE 3: Continued.

Spot identifier	Targeted GT gene	Specificity	Probe sequence (5'–3')
123	*wcxF*	Serotype12F, 12A, 12B, 44, 46	TGATTGTTTTTTTTGGACGTATCAACAAAAATAAAGGTATCAAAGAACTGC-TTGAAGCCT
124	*wcxF*	Serotype12F, 12A, 12B, 44, 46	GAAATGCTCTTCGGTTATTACTTCTAATAGAGATAGAGGAGCCTATTTTTC-TATTGAAAA
125	*wchK*	Serotype13, 14, 15B, 15C	CTTATGAAAAAATGAATCAATTGATTAAGGAATCAGATATTATCATTACCC-ATGGCGGTC
126	*wchK*	Serotype14	TAAAAATCCAATAATTGTTCCGCGGCTAAAAAAATTTGGTGAGCATGTAAA-TGATCACCA
127	*wchK*	Serotype14	AGGACAAACATTTTGAAACTTATTTGAATAACGAGAGATTTAATGTACGTT-TCAATGTGG
128	*wchL*	Serotype14, 15B, 15C	TTGTGTTGATAGTGCCTTAAAGCAAAATTTAGAATCTCTTGAAGTGATTTT-GGTGAATGA
129	*wchL*	Serotype14, 15F, 15A	AAAAATTCTTGAACAGTATGGTGATAATCCCCAAGTGATGATTTTCCATCA-AGTGAACAT
130	*wchL*	Serotype14	GCTAAGTTATTTCTTCGTAGAAGAATTGAGGAAAACAATATTGCTTTTTCG-ACTGAAATG
131	*wchL*	Serotype14, 15F, 15A, 15B, 15C	TCCTAAAATTGAGGAGAACTACTACAAGCAACATATGGATTTTAGATTTTA-TCTTGCTAG
132	*wchM*	Serotype14	AATAGAAAGTATTTTGAATCAAACGTATGATAACCTTGAGGTTCTATTAGT-CGATGATGG
133	*wchM*	Serotype14	AATAGAAAGTATTTTGAATCAAACGTATGATAACCTTGAGGTTCTATTAGT-CGATGATGG
134	*wchM*	Serotype14	CAGTATTGTAACTGGATTGTTACAATAACTGTTAGTCATTACAATGTTTTG-AATGTAGCC
135	*wchN*	Serotype14, 15F, 15A, 15B, 15C	CAAAAAAATGATATGAACATTTCGAATAAAGTTTGGATTTGTTGGTTTCAG-GGCGAAGAA
136	*wchN*	Serotype14	TATGCGAGAAAACTACTCTGGGAGTATTGGCGTAGAAAAAATAGTTTA-TGCAATTATTTT
137	*wchN*	Serotype14	GAGTTAAATAATCAATTTTCAGAAAAAAGGTGGGAACAGCTAAAACAG-ATATCGGTGTTT
138	*wchK*	Serotype13, 15B, 15C	GATGAAGTATTTATTCAAATAGGATATTCCAGTTATATTCCGAAATATTGT-GAGTGGGAA
139	*wchK*	Serotype15B, 15C	GCATGTGAATGACCATCAGCTTCAATTCGTAAAACTGACGAAAGAAAT-ATACAATTTTAT
140	*wchL*	Serotype15B, 15C	AGAAATTTTGAACCAGTACGACAGGAATTCAAGGGTTAAGATTTTTCA-TCAGCTTAATAA
141	*wchL*	Serotype15F, 15A, 15B, 15C	GAAGAAAATAATATTACTTTTTCGACTGAGATGTCACTAGGTGAAGATATG-TCATTTGTG
142	*wchM*	Serotype15F, 15A, 15B, 15C	GAAAGTATTTTGAATCAGACTTATCAAAATATCGAGATTTTATTGGTTGAT-GACGGAAGC
143	*wchM*	Serotype15F, 15A, 15B, 15C	GTACTGCAATTGGATTGTTACAGCGACTACCAATCATAGTAAGATTTTAAA-TCCTAATTT
144	*wchF*	Serotype7B, 16F, 17F, 18F, 18A, 18B, 18C, 23F, 23A, 24F, 24A, 24B, 28F, 28A, 40, 48	GAAACTTTTGTTGAAAAATTAACAGCCTTCCAACAAGATAAGGCTATCCAA-TATTATGTG

TABLE 3: Continued.

Spot identifier	Targeted GT gene	Specificity	Probe sequence (5′–3′)
145	*wchF*	Serotype16F, 17F, 18F, 18A, 18B, 18C, 23F, 24F, 24A, 24B, 28F, 28A, 48	AAGGTCTTATGGTCAAACATGCAGCTCTTTTAGTGTGTGATAGTAAGA-ATATTGAAAAAT
146	*wchF*	Serotype16F, 17F, 18F, 18B, 18C, 23A	TTCGTTACTTGAAGCATTAGCATCCACAAAGTTAAACTTACTACTCGATGT-TGGTTTTAA
147	*abp1*	Serotype17F, 24F, 24A, 24B, 48	GCCAGTCATTATCTATACCCTTGAAAAATTTCAAAATCATCCAGAAATTGA-TGAAATCTG
148	*abp1*	Serotype17F, 24F, 24A, 24B, 48	ACACAAACTCCTCATGTTTACCATCTTGATAATATTCTATCGCTTCATGAA-AAAGCATTA
149	*abp1*	Serotype17F, 24F, 24A, 24B, 48	TTATTTCTCTCTTGGAACAGAGAAAAACTTGAAAATTACGACTGTAGAAGA-TCTCGATAT
150	*wciP*	Serotype17F	GAAGAAAAAGATAGACGGATTAAATTGATTGAAAACATATCGGAATAT-CATGGAGCCTAT
151	*wciP*	Serotype17F	GTATACCAATCCTATCTCAACTTTTATGGCTCATAAGGTTTATGGATGTAA-TACGTTATT
152	*wciP*	Serotype17F	ATCTTAAAACGTATCTCGAAAATTGATGAATTAGCTAAAGATCATGCCTTG-ACTTACAAG
153	*wcrV*	Serotype17F	TCGACAGATAGTAGCAAACAGATAATTAACGAGTATCTTAATGCAGAC-AGTAGATTTAAA
154	*wcrV*	Serotype17F	CATGCAAAACTTAAGTTGTTCTGTCAGAATTTTAAGTTAGTGAGGAAA-CAGATTTTTAGG
155	*wcrV*	Serotype17F	CGATTTAATCTACTAAAAAATAACGGAGGAATGTGGGTTGACTCCACT-ATATATTTTACT
156	*wchF*	Serotype7B, 16F, 17F, 18F, 18A, 18B, 18C, 23F, 23A, 24F, 24A, 24B, 28F, 28A, 40, 48	TATAGCGTATGATATCGCTGCAATTAACAGAGCTATTGAAATTGCCAA-AGAAAATAAGGA
157	*wchF*	Serotype16F, 18F, 18B, 18C	TATAATCAGCTATTAGCAAGTACTGGATTTGATAAAGATCCACGAGTG-AAATTTGTTGGA
158	*wciU*	Serotype16F, 18F, 18A, 18B, 18C, 28F, 28A	AGAAAAAGTACAACCCGACATTATACATATTCACTCGTTTATGGGATTGCA-TAAAGAATT
159	*wciU*	Serotype16F, 18F, 18B, 18C, 28F, 28A	TCATCATCAGAGATTGACAACTGCAAATAATAAAATTAGAGTTGCTTATAT-TGGTCCAGA
160	*wciU*	Serotype18A, 18B, 18C	GACAAGGAAGATTTGTTGGCTAAAATCATCAATAATCAGTTGAAGAAA-ATTCCGCTTAAA
161	*wciV*	Serotype18A, 18B, 18C	AAATACATAACCTTTGTAGATTCAGATGACTATGTTTCTCTAGATATGCTG-CAAACTCTA
162	*wciV*	Serotype18F, 18A, 18B, 18C	AGAAGATGCTATTTTTCAAATTGATTGTTTAAAATTAGCAACATCTGCCCT-TGTTATCCC
163	*wciV*	Serotype18F, 18B, 18C	ACCCAATATCAAAATCAGTATTACGTCATTATCCAATCCATCGTTTACCTT-TTACTAAAC

TABLE 3: Continued.

Spot identifier	Targeted GT gene	Specificity	Probe sequence (5′–3′)
164	*wciW*	Serotype18F, 18A, 18B, 18C	AAGTGCAACTTGAAGATAGGGCCTACAGAATACTAAAAAAGAAATACG-GTTCTTTAATTT
165	*wciW*	Serotype18F, 18A, 18B, 18C	TGGATTGACTCAACAGTGTATTGTACAGGAATTACTACCATAGAGACA-ATTGAAAAAAAT
166	*wciW*	Serotype18F, 18A, 18B, 18C	TACGAACGCAACACCACATATAATGGTTGATGAATTAAATAATGTTTTTTC-AAAGGAACG
167	*wchO*	Serotype19F, 19B, 19C	ATAGATAGTGTAGAACAATATGTATTAGAAAAAAGACCACTACACTTG-ATGGGGGTGAAT
168	*wchO*	Serotype19F, 19B, 19C	GCTCAAAGTATTTAAGAGAGATTATCCAAATTTGATAGTTATTGGACACAG-AAATGGCTA
169	*wchO*	Serotype19F, 19A, 19B, 19C	AATTTAGAGTGGTTATTCCGTGTAGCTAATGAGCCTAAACGTCTCTTTAAA-CGTTATTTT
170	*wchO*	Serotype19A	GAGTTGCTGGAATAGACTTGATGAAACATTTACTAGAGTTGTCTAATG-AAAAAGGATACT
171	*wchQ*	Serotype19F, 19A, 19B, 19C	ATCAGATTTAGAAATTGATGTTTTGATTAACCATGAAAATGCTGGTTTTGC-TCGTGGAAA
172	*wchQ*	Serotype19F, 19A, 19B, 19C	ATCAGTAGACTATAGAAAACAGGTAGAAAACCCAATTCTTCATGGTTCTTT-TATTGTATA
173	*wchQ*	Serotype19F, 19A	GGATACAAGAGAATTTATACACCTAAAATTAGAGTTTTGCACCATCAAAAT-GTTGCAACT
174	*wciB*	Serotype20	ATACTGGGGAAAACATTTCCCAGTTAAACCCTTATTACTGTGAATTAACAG-GTTTATATT
175	*wciB*	Serotype20	AAAAAGGAAATATTATATTGAAACTCTATGTTCTCATTATGCACACACGCT-AGATGCTAG
176	*wciB*	Serotype20	AATGGCTGTTTCCGATTTTAGATTGTATGTTTGATCAGATTAATCTTTCAG-AGTTAACTG
177	*whaJ*	Serotype20	TTTCTCAAAAATTAGCGACCGAAAACTCAAATATACGAGTCTTGAAATCAG-ATAAAGGAA
178	*whaJ*	Serotype20	GATTGATGAGTACGGTTTGAAGTTTAATACGAATTTGAGAGTTTCAGA-AGATAGTGATTT
179	*whaJ*	Serotype20	CTATGTTTTTTGAGCCTATACAAAATCTATCTGTATCTAGTGTTAGCAATT-TATCGCTAG
180	*wciL*	Serotype20	GATACGTTATTATTGGGAAATGTATAGATTCTTCAAAGAATATGCATCTGA-TTATCAGGC
181	*wciL*	Serotype20	TATACATTAGACAATAAATTTGTGCTAGGTCATGTAGGACGTTTGCATTTT-CAGAAGAAT
182	*wciL*	Serotype20	GACACTACTCTCAGAAGAAGGTGTACCAAAGGAAGTAAAAATCAATGA-TAATACTTTTTT
183	*wcwK*	Serotype20	AAACAAGATATAGAGATATGGATTTGTTTCAATATTGGTTTCGAGCGG-TAGAAAACATG
184	*wcwK*	Serotype20	AATCTATTTAGCATTTTTTATTCAGGGATTATTGGTTATCATGATGCTCAT-GTCGCTATG
185	*wcwK*	Serotype20	GTGAATATGTGCCTCTGGCTTATTCAGGTAAAATTGAATCTATTATTCACA-AACAAAGA
186	*wciD*	Serotype20	TGGCTCAGAAACTGGAAAAAGAGTATTCTGGCATAGTTAGTATAATTG-ATAAAGAAATG
187	*wciD*	Serotype20	CATAAAAATTGATGAGAATATGTTCTACGTTGACATGGAGTATATTGTTTT-TCCAACTCC
188	*wciD*	Serotype20	GAGACAATTGCTAGATGTGTTACTATTATGACAAATGTTTGTCTATCAATG-GAAGATACT

Table 3: Continued.

Spot identifier	Targeted GT gene	Specificity	Probe sequence (5′–3′)
189	*whaF*	Serotype20	ACTTTAATACAAAAAACTGAATTTCCTAAATTTATCTGGACTATGTGGTGG-CAAGGAGAA
190	*whaF*	Serotype20	ATTTGGTTAGATTCAACGATGTATGTCCATCCAGATTTCCCTATTGAAATA-TTAGAAAGA
191	*whaF*	Serotype20	AGGGAAATAATAAAAAGTATCCCTAGATATTCTAGTCAAGAAGACATCTTT-TGGTTGAGA
192	*wchF*	Serotype22F, 22A	ACTTATATCGCCTATGGAACAGATACAAGCAAGTCTATTTTAAAACCTGAT-GACGAAAAA
193	*wchF*	Serotype18F, 22F, 22A, 23F	ATCGCTTTTAGAAGCTCTTGCTTCAACAAAGCTTAATTTACTGCTAGATGT-TGGCTTTAA
194	*wcwA*	Serotype22F, 22A	TAAGAAGACAAGGAGAATCGTTTTCTTTGGAATCTTATATCCGTAGTTTCT-CAGAATTAT
195	*wcwV*	Serotype22F, 22A	GAAAAACGGGGAAAAAATTAAAGTATTTTGGAGAAGGGGAATAAGATT-ATTTAGAAGTGG
196	*wcwV*	Serotype22F, 22A	GGAGAATAAGCAAAATATTCTTTATGTAGGCTCACTATCAAAAAGAAAAAA-CACAGCTCA
197	*wcwV*	Serotype22F, 22A	ACCTTATTTAAAGAACTCTCAGCTTCAATTTATTTACCCATCATCACAACT-ATTTGTGCT
198	*whaB*	Serotype22F, 22A	TGGCAGTATAGAAAGGGTAGAAGCCTTATTTGCAAATAATGACGAGAT-AGTTATAATAAA
199	*whaB*	Serotype22F, 22A	CATCATCAAAGTCCTGTTGTTGAGAAGATCAATTCTATATCTAAGGCAAAT-AAAGAACTT
200	*whaB*	Serotype22F, 22A	TTATTTTACATGGGAGTTGTGTAATTTTTTCACCATTATATGTTTCAGAGG-AGGAGTTTG
201	*wchF*	Serotype23F, 23A	CCATTTACTGGAAGAAAGATAATCTTCATGAGATTATTGAAACGAGTG-AACAAAAAACAC
202	*wchV*	Serotype23F, 23A, 23B	CCTCATTTTTGTTGACAGTGATGATTTTGTCTCTCAAGATATGGTATCTTA-TTTAGTATC
203	*wchV*	Serotype23F, 23A	GGCCAAGATATTTAAAAGAGAGTTGTTTGATGATATAAGATTTCCTGT-AGGTAAGCTATT
204	*wchV*	Serotype23F, 23A	TTTTGGAGATTACGAACACAATTATTAATCACTATGGTGATAATTTACGCG-TGTATACTG
205	*wchW*	Serotype23F, 23A	ATTTGAAACAAAATTATCAAATAAACTTGGCCTACAAAAATCTTTGCATGG-AAAGGGTGG
206	*wchW*	Serotype23F, 23A	CGGGGGGATATTATACAAAAGAGTATAAACAACTATTCAGTTCGGTAG-TAGAAAATATTA
207	*wchW*	Serotype23F, 23A	CCTATAGAGTAAATCTCCATCAATTTTTAATAAACGAGATCTCAGATGCTA-CAGTAAGAT
208	*wciB*	Serotype33F, 33A, 34, 35A, 35B, 35C, 37, 41F, 41A, 42	TTGGTTTTATCGGTGATAATACTGGCGATAATATATCCTCTCTAAATCCAT-ATTATTGTG
209	*wciB*	Serotype33F, 33A, 34, 35A, 35B, 35C, 37, 41F	ATAGTTCCAAAGAAGCGAAAGTATTATATTGAAACTCTTTATTCACATTAT-GCCCATACC
210	*wciB*	Serotype33F, 33A, 34, 35A, 35B, 35C, 37, 41F	AACTATTAGATGATTATTTACCGTGGCTTTTTTCTATTCTGGATACTATGT-ACGAACAGA
211	*wciC*	Serotype33F, 33A, 37	CAAATTTTAATATCTGATACAGATGTTTATTATTTTACTCCAGCTGGTTCA-GTAGCTGGT
212	*wciC*	Serotype33F, 33A, 37	TTACGAAATTTTATTAGAAGTTGCTAAGAAGATGGTGGGGGATGAGAA-ATATCACTTTTA
213	*wciC*	Serotype33F, 33A, 37	GTTTTACCATCGTATTATAAAGATGAAACTTTACCTATCAGTATGTTAGAA-GCAATGGCA

TABLE 3: Continued.

Spot identifier	Targeted GT gene	Specificity	Probe sequence (5′–3′)
214	*wciD*	Serotype33F, 33A, 37	AATAGCAAGACAATTCGAGAGAGAATATGAGGGAATTGTTAGAGTTAT-AAGTAAGGAAAA
215	*wciD*	Serotype33F, 33A, 37	TGCAAGAGAACAATATTCGGCTGTCTGAAAAAATGTTCTATGTAGATA-TGGAATATATTG
216	*wciD*	Serotype33F, 33A, 37	GCATAAACAAGTGATCTATCATTTGGTTGATTTTTATAATCAAATGAGATC-TAGCGCTGT
217	*wciE*	Serotype33F, 33A, 37	GCCAATTTTTAAAATCCTATAACTTTAAAGAGGTATCGCACAAGGAGA-TAGAACAAAGAA
218	*wciE*	Serotype33F, 33A, 37	CTGAATTATTTAAAAAAAGATTTTTATACTATTCGAGCAAAGACACATGAG-AGAGTGCCC
219	*wciE*	Serotype33F, 33A, 37	TAAAGTCGAAGAAAATAATCAGGAGTTGTTCTTTTTGGCAGACAATTTTTC-TAACCAGTA
220	*wciF*	Serotype33F, 33A, 37	CTGGGGAAATATGTGATGAATATGGGAAACTGTATGATAATATTCATG-TTTTCCATAAGA
221	*wciF*	Serotype33F, 33A, 37	CAGAGCGTTTGTTGAATATTAAAACAGTTGCTCATACCGATTTGCCTATAT-ATCATTATT
222	*wciF*	Serotype33F, 33A, 37	AAGGAATTGTTAGCAGCCTTAAATGCTAAAAGAGTAATTGGCTCCTTTATT-TTGAGTAAT
223	16S	*Streptococcus pneumoniae*	TATTGGAAACGATAGCTAATACCGCATAAGAGTAGATGTTGCATGACA-TTTGCTTAAAAG
224	16S	*Streptococcus pneumoniae*	ATAAGTCTGAAGTTAAAGGCTGTGGCTTAACCATAGTAGGCTTTGGAA-ACTGTTTAACTT
225	*aroE*	*Streptococcus pneumoniae*	ATTTCAAAAACGGTGTTTCAAAGGGGTTGGTATATGATATCTGCAACTA-AGAGAGTTTCTG
226	*aroE*	*Streptococcus pneumoniae*	AGCAGGGTCATCTTTTTACCTGAAATTGTAAAAGAAGGCAAGCACTTA-AAAAATCCCTTG
227	*aroE*	*Streptococcus pneumoniae*	TCAAAGGCTCTATTGTGGATGAAGGGAGAAATAGAATGCTTAATAGGA-TTGGCAACAACT
228	*ddl*	*Streptococcus pneumoniae*	TATTGAGCTCGTTGAGAAAAATCTCTCCCTTATCTGTATAGAAGAAATCGC-AACGAGATA
229	*ddl*	*Streptococcus pneumoniae*	ATGTTTGACGGCTTAGTGAAGACTGGATAAGCCAATTTTTCTTCCACTTCA-GCGATTTTA
230	*ddl*	*Streptococcus pneumoniae*	CATGACTAAATTCCTGTGTTTTGATAAAGTCACCTGACTGACTGATAAAGA-AAGTCTTGA
231	*gdhA*	*Streptococcus pneumoniae*	TGAATTCCTCCAAGCTGTTGAAGAATTTTTCAACACTTTGGAACCTGTATT-TGAAAAACA
232	*gdhA*	*Streptococcus pneumoniae*	TAAACCAAGGGATTTTGAAATTCCTCGGATTTGAACAAATCTTTAAAAACG-TCTTGACTG
233	*gdhA*	*Streptococcus pneumoniae*	TATACTGAAGAAATGCTCAAAGCTAACGGTAACAGCTTTGCTGGTAAG-AAAGTGGTTATT
234	*gdhA*	*Streptococcus pneumoniae*	ATGGACGTCTCAAAGACATCATGACCAACATCTTTAACACAGCTAAAA-CAACTTCAGAAA
235	*glcK*	*Streptococcus pneumoniae*	AAATGGTCAATCAAGACCAACATTTTGGATGAGGGAAGTCATATCGTT-GATGATATGATT
236	*glcK*	*Streptococcus pneumoniae*	AACAAAAGATTGAAAAAGCTTTGGGCATTCCATTTTTCATCGATAATGATG-CCAACGTAG
237	*glcK*	*Streptococcus pneumoniae*	AGAATTCCTTCTACAAGGTGTTCAAAAAGTTTACGATGAAAATAGTTTCCC-ACAAGTACG
238	*spi*	*Streptococcus pneumoniae*	AGAAGGTATTCTCCTTCTGGAACAGTAAAGCTAAAGTTGGTGTTGTAG-TTGACATCAACT
239	*spi*	*Streptococcus pneumoniae*	TGATATAGTCTGCTAGATAAGGCTCGTCCGTTTCTTTGTCATTGATGTAGA-GTTTATCAT
240	*spi*	*Streptococcus pneumoniae*	AACATTGCTCCAAAAAAAGATACGGCTCAAAGCTAGTAATGACAGAAT-CAGGAGGAATAA
241	*tktA*	*Streptococcus pneumoniae*	ACAATTTTTACAAGATTTTCTACAGTAAAGCCATATTCTGCCAATACTTTT-GGTGCTGGG

TABLE 3: Continued.

Spot identifier	Targeted GT gene	Specificity	Probe sequence (5′–3′)
242	*tktA*	*Streptococcus pneumoniae*	CAATCAAGATGGTATCAAAGTCGGCTGCATTTTCATATACAACATAAG-CACCTTTAGCAA
243	*tktA*	*Streptococcus pneumoniae*	TTCAAGATTGTTCCCATTGCAAATTCACGAACACCAAACTGAATGTTACGA-TTCAAGCGA
244	*tktA*	*Streptococcus pneumoniae*	AACATGTTCTTTGAAATCAGCATATACTTGTTCTGGAATTTCAAATGGTTC-GTAGTCCCA
245	*tktA*	*Streptococcus pneumoniae*	TCTCCACAGATAACGTAAGTATAGTGGTCAAAGATATTGTAGCCTTCA-CGGTTATATTTG
246	*xpt*	*Streptococcus pneumoniae*	AGATTCCTTTTTAACCCACCAAGTTGACTTTAGCTTGATGCGAGAGAT-TGGTAAGGTTTT
247	*xpt*	*Streptococcus pneumoniae*	ATGATTTTCGCCAAAAAAGCTAAGAACATCACCATGAACGAAGGCATC-TTAACTGCTCAA
248	*xpt*	*Streptococcus pneumoniae*	TTTGATTATCGACGATTTCCTTGCTAATGGCCAAGCTGCTAAAGGCTTGAT-TCAAATCAT
249	KP_gapA	*Klebsiella pneumoniae*	GACGTTGTTGCTGAAGCAACCGGTATCTTCCTGACCGACGAAACCGCT-CGTAAACACATC
250	KP_rpoB	*Klebsiella pneumoniae*	AACGGTGTGGTTACTGACGAAATTCACTACCTGTCTGCTATCGAAGAA-GGCAACTACGTT
251	KP_mdh	*Klebsiella pneumoniae*	TGTACGATAAAAACAAACTGTTCGGCGTTACCACGCTGGACATCATCC-GTTCCAATACCT
252	KP_pgi	*Klebsiella pneumoniae*	CCTGGCCTTTGGTAAATCCCGCGAAGTGGTTGAGCAGGAATATCGCGA-TCAGGGTAAAGA
253	SA_arcC	*Staphylococcus aureus*	TGATAGGCTATTGGTTGGAAACTGAAATCAATCGCATTTTAACTGAAA-TGAATAGTGATA
254	SA_aroE	*Staphylococcus aureus*	AAGTTTTGATTGGTCATTAGTTCCTGGTTATATTGTTGCTCAAATGTTAGG-TGCAATTGT
255	SA_glpF	*Staphylococcus aureus*	TAAGAATTACTTTGCCAACTTTTTAAGTGAGATTATCGGAACAATGGCATT-AACTTTAGG
256	SA_gmk	*Staphylococcus aureus*	CGTAGATTACTTTTTTAAAACTAGGGATGCGTTTGAAGCTTTAATCAAAGA-TGACCAATT
257	LP_acnF	*Legionella pneumophila*	CGAAAAAAGGGGTTGTTGGTAAATTTGTTGAATTTTATGGTCCTGGA-CTTAATGATTTA
258	LP_mompS	*Legionella pneumophila*	TCAATGTGAACTGGTATCATTTTGATAACGACAGTGATCACTGGTTTGATT-TTGCTAACT
259	CP_groES	*Chlamydophila pneumoniae*	TTCTTTACCTGCGTTACTTGCAATTTGCTTTAATGGAGCTGTTAATGCTTT-TAGAATAAT
260	CP_gyrA	*Chlamydophila pneumoniae*	GTTTGGTGGCTAAAAATAAGAAGCCGGCATTATCAAAATTCTTAATATTCA-ATATAGCTG
261	CP_gyrB	*Chlamydophila pneumoniae*	CCAAGACCTTTATACCTCTGAATTTCTATGCCTTTTCTTCCAAGATTTTTA-AGATAGTTA
262	CP_dnaA	*Chlamydophila pneumoniae*	CCTGCTGCTTCTAAAACATCTTTTAAAAGAGTTTTCACATCATCTTCATAT-AGTAATTGG
263	CP_accA	*Chlamydophila pneumoniae*	TGATAACAGTATCGATAATGCCAAATTGTTTTAAGTTTTCTCCATGCATTT-TCAACATGG
264	CP_dnaK	*Chlamydophila pneumoniae*	TCAAAAAACAAGAAGGCATTGATCTTAGCAAAGATAATATGGCCTTAC-AAAGACTTAAAG
265	MP_gyrB	*Mycoplasma pneumoniae*	AGGAACCTTTATTTGAGGACATTATCTTTGGTGAAAAAACCGATACTGTTA-AATCAGTTA
266	MP_gyrA	*Mycoplasma pneumoniae*	ACAAGATCAAATTGACAAAATTCGTCAGGAATTAGCACAATCAGCAAT-TAAAAACATCTC
267	MP_dnaJ	*Mycoplasma pneumoniae*	TTGCGCAAGCTCAAGGAATTTATTAAACCTAATCAAGAGGTAAAACAATAT-TTAAACGCA

TABLE 3: Continued.

Spot identifier	Targeted GT gene	Specificity	Probe sequence (5′–3′)
268	MP_lgt	*Mycoplasma pneumoniae*	TGGGATTGCCTTTGGCATCTTAATGTTTGTCTTGAAGTTAATTTACTTTTA-CAAGATTCA
269	MP_fus	*Mycoplasma pneumoniae*	TAAGCTTCCGTGAAACCTTCAATAAAGAAAGTGAAGTTGAGGGTAAAT-ACATTAAACAAT
270	MP_lspA	*Mycoplasma pneumoniae*	TTTGAAGAACTGAATTAAAAAGGAGAGGAACAGACCAATAAAACTAAA-GGTAATGCAACA
271	PA_trpE	*Pseudomonas aeruginosa*	TCACCGAAAAAATGGTGATCGAACGTTACTCCAACGTCATGCACATCG-TGTCCAACGTCA
272	PA_nuoD	*Pseudomonas aeruginosa*	GATCATGATGGCGGAGTTCTTCCGTATCCTGAACCACCTGCTGTACCT-GGGCACCTATAT
273	SP_gki	*Streptococcus pyogenes*	ATTCAGCCATCAAAGCAGCTATTGACAATGGTGAAGGTGTTACCAGTA-AAGACATTTTCA
274	SP_xpt	*Streptococcus pyogenes*	ATCGCTGGTAAATTCCTATCTAAAGAAGACAAGGTTTTGATTATTGATGAC-TTTTTAGCT

diagram of the probe positions on the microarray is shown in Figure 1(a).

3.2. Evaluation of the Microarray. A total of 274 oligonucleotide probes were used in this microarray, including positive and negative controls and GT gene-specific probes. The microarray probes were tested using 36 pneumococcal isolates from 23 vaccine-associated serotypes and 19 additional pneumococcal isolates belonging to other serotypes (Table 1). Figure 1(b) shows the examples of scanned pictures of 6 strains representing different serotypes. Examples of the same serotype were tested repeatedly and shown to have an identical signal pattern, for example, 5 times for serotype 3 (data not shown). Of 23 strains representing 23-valent vaccine serotype, 18 strains hybridized to all the specific set of probes, and four strains hybridized to almost all the specific set of probes (Table 4). The strain representing serotype 22F may actually belong to serotype group 22F/22A, since this sample failed to hybridize specifically to *wchF* and *wcwA* probes but hybridized to the rest of group 22F/22A specific probes. Of the 13 strains representing the 23 vaccine-related serotypes, only 1 isolate (serotype 46), failed to hybridize to a specific probe while the other 12 strains hybridized perfectly. Of the 20 nonvaccine serotypes, 19 strains either hybridized partially to GT-specific probes or did not hybridize to any probes. One strain, representing serotype 23A, hybridized to most of the 23F-specific probe; thus, 23A may be indistinguishable from 23F using GT gene sequences.

4. Discussion

In order to develop a more effective *S. pneumoniae* vaccine, simple detection methods are required to serotype large numbers of clinical isolates. Conventional serotyping methods using large panels of antisera are labourious and require technical expertise. Our microarray method can determine serotype of a strain at one time and needs no expertise.

In addition, the microarray method described here has the potential to be automated. To our knowledge, our report describes the first microarray to utilize GT genes to predict serotype of any bacteria.

Several molecular typing methods have been developed based on serotype-specific sequences [12–21]. Wang et al. [21] described microarray method using *wzy* and *capA* genes. Our approach is different in that GT genes were selected as serotype-specific genes. Since GTs catalyze the transfer of the sugar moiety to an acceptor and generate a serotype-specific capsular polysaccharide, detecting GT genes can directly reflect polysaccharide structure. We discovered considerable variability within *S. pneumoniae* GT genes, which provides groundwork for future investigations into new *S. pneumoniae* capsular types. Our method using GT genes can not only discriminate serotypes but can give information of the capsular polysaccharide structure.

The DNA microarray described here accurately detects the majority of *S. pneumoniae* serotypes and serogroups included in the 23-valent vaccine and in the 7, 9, 11, 13-valent conjugate vaccines, which will permit serotype surveillance before and after vaccination. Since 1983, the 23-valent pneumococcal vaccine has been administered to persons in the United States aged >2 years with certain underlying medical conditions or aged >65 years. In 2000, the more effective PCV7, 7-valent pneumococcal conjugate vaccine, which protects against serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F was approved for administration [22]. As a result of PCV7, antibiotic-resistant invasive pneumococcal infections have decreased dramatically in young children and older persons [23]; however, an increase in disease associated with serotypes not included in the PCV7 vaccine, has been observed [24, 25]. To address serotype vaccine coverage, the Advisory Committee on Immunization Practices (ACIP) issued recommendations in February 2010 for a newly licensed 13-valent pneumococcal conjugate vaccine (PCV13), which contains the seven serotypes in PCV7 (4, 6B, 9V, 14, 18C, 19F, and 23F) and six additional serotypes (1, 3, 5, 6A, 7F, and 19A) [26]. Taken together, our DNA

84	67	80	66	51	43	50	42	27	19	26	18	77		76	61	57	60	56	37	31	36	24	13	6	12	5
85	65	70	64	47	41	46	40	25	17	22	16	75		74	59	55	49	54	35	23	34	29	11	4	10	3
69	63	68	62	45	38	44	38	21	15	20	14	72		85	48	53	58	52	33	29	32	28	9	2	8	1
155	149	154	148	130	122	129	121	104	98	103	97	158		157	140	133	139	128	116	110	115	109	92	86	91	71
153	147	151	143	127	120	126	119	102	96	101	95	156		146	138	125	137	134	114	108	113	107	90	83	89	82
151	142	150	141	124	118	123	117	100	94	99	93	145		144	136	132	135	131	112	106	111	105	88	81	87	78
P	218	P	217	200	194	199	193	177	171	176	169	P		P	212	206	211	205	189	183	188	182	166	160	165	159
P	220	P	219	202	196	201	195	179	173	178	172	P		P	214	208	213	207	191	185	190	184	73	162	170	161
P	222	P	221	204	198	203	197	181	175	180	174	P		P	216	210	215	209	192	187	7	186	168	164	167	163
N	N	N	N	N	N	N	N	P	P	P	P	N		N	N	N	N	N	N	N	N	N	P	P	P	P
E	E	E	E	E	E	N	N	N	P	N	P	N		E	E	E	E	E	E	N	N	N	P	P	P	P

(a)
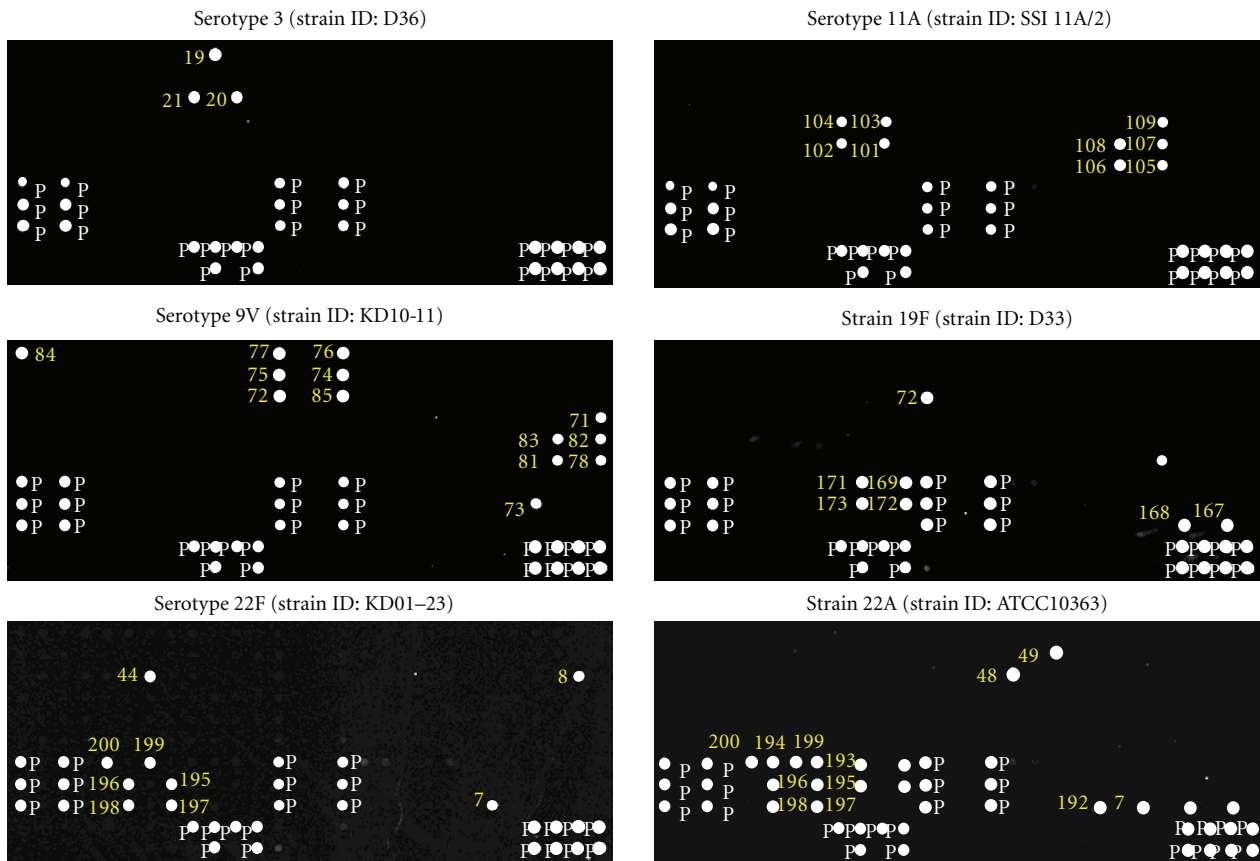
(b)

FIGURE 1: (a) Microarray oligonucleotide probes layout. Oligonucleotides 1 to 222 are provided in Tables 2 and 3. P represents *S. pneumoniae* housekeeping genes and 16S rDNA positive control oligonucleotides. N indicates negative control oligonucleotides designed from housekeeping genes of other bacterial species. E denotes empty spot. (b) Scanned microarray images of *S. pneumoniae* genomic DNA hybridized with 6 samples (serotype 3, 9V, 11A, 19F, 22F and 22A). The numbers correspond to the spot identifiers given in Tables 2 and 3, and Figure 1(a) P indicates positive spot.

TABLE 4: Microarray results of each strain.

Serotype	Strain ID	Positive probe[a]	Microarray result	Assined group
1	ATCC6301	1, 2, 3, 4, 5, 6	Perfectly matched	1
2	ATCC6302	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18	Perfectly matched	2
3	D36	19, 20, 21	Perfectly matched	3
4	JHK27	22, 23, 24, 25, 26, 27, 28, 29, 30	Perfectly matched	4
5	ATCC6305	23, 24, 31, 32, 33, 34, 35, 36, 37	Perfectly matched	5
6B	MSC1047	38, 39, 41, 42, 43	1 probe of group 6A/6B did not hybridized	6A/6B
7F	ATCC10351	44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58	Perfectly matched	7F/7A
8	ATCC6308	59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70	Perfectly matched	8
9V	KD10-11	71, 72, 73, 74, 75, 76, 77, 78, 84, 85, 81, 82, 83	Perfectly matched	9A/9V
9N	KD01-26	71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83	Perfectly matched	9L/9N
10A	ATCC8334	86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100	Perfectly matched	10A
11A	SSI11A/2	101, 102, 103, 104, 105, 106, 107, 108, 109	Perfectly matched	11A/11D
12F	ATCC6312	11, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124	1 extra probe of group 2 hybridized	12F/12A/12B/44/46
14	D59	101, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137	Perfectly matched	14
15B	ATCC10354	125, 128, 131, 135, 138, 139, 140, 141, 142, 143	Perfectly matched	15B/15C
17F	ATCC6317	144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156	1 extra probe of group 18B/18C hybridized	17F
18C	ATCC10356	156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166	Perfectly matched	18B/18C
19F	D33	72, 167, 168,169, 171, 172, 173	Perfectly matched	19F
19A	D4	71, 73, 74, 169, 170, 171, 172, 173,	1 extra probe of group 19F hybridized	19A

23 serotypes included in 23-valent vaccine

TABLE 4: Continued.

Serotype	Strain ID	Positive probe[a]	Microarray result	Assined group
20	ATCC6320	174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191	Perfectly matched	20
22F	KD01-23	7, 8, 44, 195, 196, 197, 198, 199, 200	5 probes of group 22F/22A did not hybridized and 1 extra probe of group 7F/7A hybridized	22F/22A
23F	KD11-15	144, 145, 156, 193, 201, 202, 203, 204, 205, 206, 207	Perfectly matched	23F
33F	ATCC10370	208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222	Perfectly matched	33F/33A/37
Other serotypes included in 23 groups				
6A	MSC1943	38, 39, 40, 41, 42, 43	Perfectly matched	6A/6B
7A	ATCC6307	44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58	Perfectly matched	7F/7A
9A	ATCC8333	71, 72, 73, 74, 75, 76, 77, 78, 84, 85, 81, 82, 83	Perfectly matched	9A/9V
9L	ATCC10349	71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83	Perfectly matched	9L/9N
11D	SSI11D/1	101, 102, 103, 104, 105, 106, 107, 108, 109	Perfectly matched	11A/11D
12A	SSI12A/5	110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 274	Perfectly matched	12F/12A/12B/44/46
12B	SSI12B/1	110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 274	Perfectly matched	12F/12A/12B/44/46
15C	SSI15C/2	125, 128, 131, 135, 138, 139, 140, 141, 142, 143	Perfectly matched	15B/15C
18B	ATCC10355	156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166	Perfectly matched	18B/18C
22A	ATCC10363	7, 48, 49, 192, 193, 194, 195, 196, 197, 198, 199, 200	Perfectly matched	22F/22A
33A	ATCC8340	208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222	Perfectly matched	33F/33A/37

TABLE 4: Continued.

Serotype	Strain ID	Positive probe[a]	Microarray result	Assined group
44	SSI44/3	110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 274	Perfectly matched	12F/12A/12B/44/46
46	SSI46/2	110, 111, 112, 113, 114, 115, 116, 117, 119, 120, 121, 122, 123, 124, 274	1 probe of group 12F/12A/12B/44/46 did not hybridized	12F/12A/12B/44/46
7B	ATCC10348	143, 155	Partial hybridization	Not included in 23 group
7C	ATCC10350	none	None hybridization	Not included in 23 group
10F	ATCC6310	86, 87, 88	Partial hybridization	Not included in 23 group
10B	SSI10B/2	71, 72, 73, 74, 78, 79, 80, 81, 82, 83	Partial hybridization	Not included in 23 group
10C	SSI10C/2	71, 72, 73, 74, 75, 76, 77	Partial hybridization	Not included in 23 group
11F	ATCC6311	103, 104, 105, 106, 107, 108, 109	Partial hybridization	Not included in 23 group
11B	SSI11B/2	101	Partial hybridization	Not included in 23 group
11C	ATCC10353	101, 274	Partial hybridization	Not included in 23 group
15F	ATCC6315	101, 129, 131, 135, 141, 142, 143	Partial hybridization	Not included in 23 group
15A	ATCC6330	101, 129, 131, 135, 141, 142, 143	Partial hybridization	Not included in 23 group
17A	SSI17A/2	none	None hybridization	Not included in 23 group
18F	ATCC6318	144, 145, 156, 157, 158, 159, 162, 163, 164, 165, 166, 193	Partial hybridization	Not included in 23 group
18A	ATCC10344	144, 145, 156, 158, 160, 161, 162, 164, 165, 166	Partial hybridization	Not included in 23 group
19B	ATCC10358	72, 167, 168,169, 171, 172	Partial hybridization	Not included in 23 group
19C	ATCC10359	72,169,171,172	Partial hybridization	Not included in 23 group
23A	KD12-06	144, 146, 156, 201, 202, 203, 204, 205, 206, 207	1 probe of group 23F did not hybridized	23F
23B	ATCC10364	7, 46, 202,	Partial hybridization	Not included in 23 group
33B	ATCC10342	none	None hybridization	Not included in 23 group
33C	ATCC8339	none	None hybridization	Not included in 23 group
33D	SSI33D/2	49, 57	Partial hybridization	Not included in 23 group

(Serotypes not included in 23 groups)

Explanatory notes: [a]The numbers correspond to the spot identifiers given in Tables 2, 3, and Figure 1(a).

microarray will be able to monitor serotype prevalence of all vaccine-related serotypes. However, in examining serotype replacement in vaccinated population a further study to distinguish more than 90 serotypes is required and is currently under investigation. Moreover, further study of the reproducibility of the microarray is needed.

5. Conclusion

We developed a *S. pneumoniae* DNA microarray that identifies GT gene polymorphisms to distinguish capsular types. We believe that our microarray system is more reliable and cost-effective and will help to survey the emergence of new *S. pneumoniae* serotype.

Acknowledgment

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