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-γ) for 24 h at 37°C in 5% CO₂. 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 × SSC/0.2% SDS for 15 min, transferred to a second beaker filled with 2 × SSC/0.2% SDS to incubate for 5 min at 95°C, rinsed three times with dH₂O, 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 dH₂O and 20 µL of 2.5 × 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 glycéron 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 × 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 × SSC/0.1% SDS solution for 3 min, 0.05 × SSC for 3 min, and 95% ethanol for 90 s at room temperature. The washed microarray slide was dried by centrifugation and scanned 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*) |
|------------|-----------------------------------------------|
| 1          | wchB (1, 2, 3) wchD (4, 5, 6) wchH (13, 14, 15) wchI (16, 17, 18) |
| 2          | wchF (7, 8, 9) wchG (10, 11, 12) wchI (16, 17, 18) |
| 3          | wchE (19, 20, 21) wchI (22, 23, 24) wchJ (31, 23, 24) |
| 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) |
| 10         | wciB (86, 87, 88) 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/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, 134) 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) abpI (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, 70, 73, 74) wchQ (171, 172, 173) |
| 20         | wciB (174, 175, 176) whaJ (177, 178, 179) wciL (180, 181, 182) wcwK (183, 184, 185) wcwV (186, 187, 188) whaB (189, 190, 191) |
| 22F/22A    | wchF (7, 8, 192, 193) wcwA (48, 49, 194) wcwV (195, 196, 197) whaB (198, 199, 200) |
| 23F        | whcF (144, 156, 145, 193, 201) wcwA (202, 203, 204) wcwV (205, 206, 207) |
| 33F/33B/37 | wciB (208, 209, 210) wciC (211, 212, 213) wciD (214, 215, 216) wciE (217, 218, 219) wciF (220, 221, 222) |

**Explanatory notes:** *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
| Spot identifier | Targeted GT gene | Specificity | Probe sequence (5’–3’) |
|----------------|-----------------|-------------|----------------------|
| 1              | wchB             | Serotype 1  | ATAAGATTATGAGAAAAATATAGACCGGATGTCTTGACATATACC-GTGAACCCAAAAT |
| 2              | wchB             | Serotype 1  | TTTATTGGTggAGATATTAAAAAGGTAGATAGACTTCTGGCTGCT-GCCCAACAAAT |
| 3              | wchB             | Serotype 1  | GAAAAATGAAAAACGAAAAAGATTGGAGCTTCAAGGGAGAATTGATATA-GAGCAAAATTT |
| 4              | wchD             | Serotype 1  | TTAATGGAAGGATGATGACATCTATGTTTGGATATGCTCTCATA-TAAATTGAGAT |
| 5              | wchD             | Serotype 1  | GCCATAGATTTGTATGGAAGCACAGATGATATCGAAATATTACAGTTTGGG |
| 6              | wchD             | Serotype 1  | AGGGAATTCGGGGAATCTCAATAATGATGAAATGAAAAAGATGAGGAAATGAGCAGTTTCG |
| 7              | wchF             | Serotype 1  | TTTTTGAGAAATACAAGAATATCACAAGATGGAACAGAATAGTTTACAGTTTCG |
| 8              | wchF             | Serotype 1  | CTTAAAAAGACCTTTTGTCCAATACACATGGAACAGAATAGTTTACAGTTTCG |
| 9              | wchF             | Serotype 1  | TTAATGGAAGGACATGGAAGGAGAATACGCATTTCGAGACTTAG-TAAATTAACCTTA |
| 10             | wchG             | Serotype 2  | GCAAATACCAAGAAAAATACCTTAAAAATTAAGTTATACAGATTTCCCTCCTGCTTGAAGAG |
| 11             | wchG             | Serotype 2  | TAGAAATTTAAACATCTGTGATGTATAGATATCGATAGCTATGAGATAGTTTGAGAT |
| 12             | wchG             | Serotype 2  | TTAATCGAAATCTCTCAAGTAATGAGGAGACAGATATTATCGTGAATTTATACAGTTTCG |
| 13             | wchH             | Serotype 2  | TAGAAACAGAAAATTTTTATCGGATAAAAAGCTTCTTTGGGGAATACCTCTAAAAACG |
| 14             | wchH             | Serotype 2  | CGTAATACGAAAAAGTTACCCTGATACCTTAAATGTGTTGAATTAATCTCGGAAAGAAAA |
| 15             | wchH             | Serotype 2  | CTTTTGTTGGAACCTCTCTAAATGTGAACAGATATTGTTGAGATGTTATTTACAGTTTCG |
| 16             | wchI             | Serotype 2  | CATTTTACGAGAACATGGAAATGTGGAGATGAGCTTTGAAACAAAAAGG-AATTAATACCTA |
| 17             | wchI             | Serotype 2  | TGATTATTGAGAGGATGAGCTTACATCTTCTGAGAGATATAAACAGATTTTAAATTTAAG |
| 18             | wchI             | Serotype 2  | TACAAAAAGAGATAATTACTTACTACAGAGAAAAACAGATATCTGTGAAACCCAGGAGATATATATAT |
| 19             | wchI             | Serotype 2  | TATAAGTCTCTAGAGATGTGAGATGGAGAATTTATATGAGGTCTGCTGATATTACCTC |
| 20             | wchI             | Serotype 2  | ACCTTTAAAAAGGCTTAAACACTGTTATGCAGGATATCTCTGGTGTATGACAGATGTCA |
| 21             | wchI             | Serotype 2  | ACCTTTAAAAAGGCTTAAACACTGTTATGCAGGATATCTCTGGTGTATGACAGATGTCA |
| 22             | wchI             | Serotype 2  | ACCTTTAAAAAGGCTTAAACACTGTTATGCAGGATATCTCTGGTGTATGACAGATGTCA |
| 23             | wchI             | Serotype 2  | ACCTTTAAAAAGGCTTAAACACTGTTATGCAGGATATCTCTGGTGTATGACAGATGTCA |
| 24             | wchI             | Serotype 2  | ACCTTTAAAAAGGCTTAAACACTGTTATGCAGGATATCTCTGGTGTATGACAGATGTCA |
| 25             | wchI             | Serotype 2  | ACCTTTAAAAAGGCTTAAACACTGTTATGCAGGATATCTCTGGTGTATGACAGATGTCA |
| 26             | wchI             | Serotype 2  | ACCTTTAAAAAGGCTTAAACACTGTTATGCAGGATATCTCTGGTGTATGACAGATGTCA |

Table 3: Oligonucleotide probes used in this study.
| Spot identifier | Targeted GT gene | Specificity | Probe sequence (3′–5′) |
|----------------|-----------------|-------------|------------------------|
| 27             | wciK             | Serotype4   | GTGAAGATACCTATATAGAAAAAGTGTCAATAGAAGATGTTTGGTTTCTGTATACCTA |
| 28             | wciL             | Serotype4   | AAAAGCCCTCTACATCGTTTCTCTCTGCTAGAATAATAAAGAAAGGAGATTGATATA |
| 29             | wciL             | Serotype4   | AGAATCATTATTTAATCCAAACAAATTTGTATTTTACTCTTTATGTTGAATTGAGGGTGAGTA |
| 30             | wciL             | Serotype4   | AAAAACATTAGTACTTTACTCTATCACGAGAGCTGTGTGCTATAGGAAAAAGATAAGTA |
| 31             | wciL             | Serotype5   | TTACATAGGATATTTAATTATTTTTGAGATTTTTGGTTTCGTTTTACCTA |
| 32             | whaC             | Serotype5   | TTTCTGACTCTCAAGATATGAGATGTATATTCAAGAAAGAGACGCTATAAAGGAAAAAGTTGTTT |
| 33             | whaC             | Serotype5   | TATATCCGAACTCCTCAACTTTTGAACCTTTTAAAGGAAATATCATATCCGTTCAGATTATT |
| 34             | whaC             | Serotype5   | GAAGACATAAATCTTCAGCTGATAGGGAATGAAAGCGCTATAAAGGAAAAAGTTGTTT |
| 35             | whaD             | Serotype5   | AAGAGGGAGCTAGCTTTGCTATAGGGAATGAAAGCGCTATAAAGGAAAAAGTTGTTT |
| 36             | whaD             | Serotype5   | GAGGTTTCTCAAGGATATGATTTCAGAAGTAAGGAAAAAGTTGTTT |
| 37             | whaD             | Serotype5   | TCTAAATACATATAATCTCTCTTTTCTAGAAGAGGAGATATCTTCAGAGTTGATGAGGTTT |
| 38             | wciN             | Serotype6A, 6B, 33D | AATAGATTATCAGAAAATTTGCGCAGAGAAATTTGAGATAGAGAAGGTTGAGGTG |
| 39             | wciN             | Serotype6A, 6B, 33D | TTACAGGAAGATTAGGATGTTTAAATGCAAGTTTTTATATAACTCATTTGCTGTACTTCTT |
| 40             | wciN             | Serotype6A, 6B, 33D | GAAGACAGTCATATGAGGCAATAGGGAATGAAAGCGCTATAAAGGAAAAAGTTGTTT |
| 41             | wciP             | Serotype6A, 6B, 33D | GGACACCTTTTTATTAGGAGTATTGAAGTAAGGAAAAAGTTGTTT |
| 42             | wciP             | Serotype6A, 6B, 33D | CAGGTTTTTATACATGCTATTGCTAGAGATTCTCTTTGCTATAGGGAATGAAAGGTTGTTT |
| 43             | wciP             | Serotype6A, 6B, 33D | CTTACACATGTGCTGTTTAAATCGAAGTTCTCTTTGCTATAGGGAATGAAAGGTTGTTT |
| 44             | wchF             | Serotype7F, 7A | ATACAATCAATGTGTGTGTGTGTTGAGAATAATCTCGATTTCTGAGTTGAGGTTT |
| 45             | wchF             | Serotype7F, 7A | AAAAATAATGCTCAACAAGAGATTATAAGCACTCAGAGAAGGACACCTATATTGCTATAG |
| 46             | wchF             | Serotype7F, 7A | TTGTTACAGGAACTGTTTTGCTAGAAGATGTTTGAATTGGAATGAAAGGTTGTTT |
| 47             | wcwA             | Serotype7F, 7A | AAGTGCTATGTTCTCTTTCGTTGAAAGGTTATATGTTGGAATGAAAGGTTGTTT |
| 48             | wcwA             | Serotype7F, 7A | ATGTTTGTTGAGATTACCGATTATCAAGGAAATCTCTCTAATCTCAAGGAGGTTTAGAAT |
| 49             | wcwA             | Serotype7F, 7A | CTTACAAATGCTACCAAGGAGATGTTAGCAGACTGAGCTTTTATATTGTTGGAATGAAAGGTTGTTT |
| 50             | wcwA             | Serotype7F, 7A | AAATATGAGAATATTACCAAGGATGTTAGCTAGAGAGGACACCTATATTGCTATAG |
| 51             | wcwF             | Serotype7F, 7A | TATTTTATTGGGAGATGAGGTTGCTACTTACCCAAGTAGGGTTTGGAGATTATGTTGGAATGAAAGGTTGTTT |
| 52             | wcwF             | Serotype7F, 7A | ATGAAAATTGATGAGGAAAAATCGAGAGAAGACTCAGACCTTTTTTATGAGGAGAGTAATGTTGGAATGAAAGGTTGTTT |
| Spot identifier | Targeted GT gene | Specificity | Probe sequence (5′–3′) |
|-----------------|-----------------|-------------|-----------------------|
| 53              | wcwG Serotype7F, 7A | AAAACGATTACCCGGGATTTTATCCATAATTTTGGTTTAGAGAATGGTGTC-TAGAAAAAT | |
| 54              | wcwG Serotype7F, 7A | GGTGCAGATAGAAGAGGAGTGGTCTAACCCTTCTTAGAAGAAATGCAATTTTT-ATAGTTTTAAT | |
| 55              | wcwG Serotype7F, 7A | ATAAAAAAGGAGACCTGGTCTAACCTATGAGCAATGGAATCCATTTTTTTAT-ATAGTTTTAAT | |
| 56              | wcwH Serotype7F, 7A | GGAACAGAGCTTAGTAAAGAATGGTATGTTATATCATGAAG | |
| 57              | wcwH Serotype7F, 7A | ATTTTGCTAAATCTAGAAAGCGGCAATGTCCCAATAAAAGGACATGTT-ATAGTTTTAAT | |
| 58              | wcwH Serotype7F, 7A | TATTTTGAAATCTATAGACAGTATGTTATGTTATATCATGAAG | |
| 59              | wciO Serotype8 | AACAAATGAGCTTGAAGAAGGACATGATGTTATGTTATATCATGAAG | |
| 60              | wciO Serotype8 | TAAAGCCTTGAATTAAGAAGAAGAAGGACATGATGTTATGTTATATCATGAAG | |
| 61              | wciO Serotype8 | ATGCTTTGATATGCAATTGATGGCGAGAATGCACTTTTATCTTCGATGAAATAGGTTGTTT-GCTCAGATTGA | |
| 62              | wciO Serotype8 | AAAATGAGCTTGAAGAAGGACATGATGTTATGTTATATCATGAAG | |
| 63              | wciO Serotype8 | TAAAGCCTTGAATTAAGAAGAAGAAGGACATGATGTTATGTTATATCATGAAG | |
| 64              | wciO Serotype8 | ATGCTTTGATATGCAATTGATGGCGAGAATGCACTTTTATCTTCGATGAAATAGGTTGTTT-GCTCAGATTGA | |
| 65              | wciO Serotype8 | AAAATGAGCTTGAAGAAGGACATGATGTTATGTTATATCATGAAG | |
| 66              | wciO Serotype8 | TAAAGCCTTGAATTAAGAAGAAGAAGGACATGATGTTATGTTATATCATGAAG | |
| 67              | wciO Serotype8 | ATGCTTTGATATGCAATTGATGGCGAGAATGCACTTTTATCTTCGATGAAATAGGTTGTTT-GCTCAGATTGA | |
| 68              | wciO Serotype8 | AAAATGAGCTTGAAGAAGGACATGATGTTATGTTATATCATGAAG | |
| 69              | wciO Serotype8 | AAAATGAGCTTGAAGAAGGACATGATGTTATGTTATATCATGAAG | |
| 70              | wciO Serotype8 | AAAATGAGCTTGAAGAAGGACATGATGTTATGTTATATCATGAAG | |
| 71              | wciO Serotype9A, 9V, 9L, 9N, 19A | ATTAACGATAGAAGAAACAGTGATGTCTGTGATGAAATAGTATGTTATGTT-ATTGAATATAGAT | |
| 72              | wciO Serotype9A, 9V, 9L, 9N, 19B, 19C | TTTTGATGTTATTCAGACACATAAAGGAGCTCCATTATTTGATGGAATGAAAAATTGAATCT | |
| 73              | wciO Serotype9A, 9V, 9L, 9N, 19A | GAAAGAATATATTATACATTCATCAATGATAATGGAATTAATGCTGTGT-TATGGAATCT | |
| 74              | wciO Serotype9A, 9V, 9L, 9N, 19A | GAGTAGGGGTATTTGTAGATGTCTTCTTGTAGTGTTGCTCATAATAAAAAGGATATT | |
| 75              | wcjA Serotype9A, 9V, 9L, 9N, 19A | AACAGGTGGCTATGAGGAGGAAACACTTTTATCAAAGGGAGCTCAACATATAATTTTAT | |
| 76              | wcjA Serotype9A, 9V, 9L, 9N, 19A | TTTAAAAAGGCAATTTTGATGAGGACTTGTGCCATGATGATTGTTGCTTCATCTCATTTTATATTGAATATAGAT | |
| 77              | wcjA Serotype9A, 9V, 9L, 9N, 19A | GAGTAGGGGTATTTGTAGATGTCTTCTTGTAGTGTTGCTCATAATAAAAAGGATATT | |
|                |                |             |                       |
Table 3: Continued.

| Spot identifier | Targeted GT gene | Specificity | Probe sequence (5′–3′) |
|-----------------|------------------|-------------|------------------------|
| 78              | wcjB              | Serotype9A, 9V, 9L, 9N | GAAAAGCTAATTTTAGAAAAATGAACTAATTGTGTTTCAGAAACCTTACCCAGCGCTTT |
| 79              | wcjB              | Serotype9L, 9N          | AGTTGTTCTAGTTGTAGCAGATCATCTTATTCTGCGAAATCATATAAGAAAAGTGAAGCTT |
| 80              | wcjB              | Serotype9L, 9N          | CAATCCGAGGAGAGTTTGTATATTTGAAATACGCATATGATAACAAAAGTGAATAGAGT |
| 81              | wcjC              | Serotype9A, 9V, 9L, 9N  | AAAATTTTCAGTTGAACACTTTGTAAAGAGGAAGAGCATAGGATTGTGCA-AATCTCTGATGT |
| 82              | wcjC              | Serotype9A, 9V, 9L, 9N  | TTATCAAATAAAGAGTTTATTAAACCATCTTCTCAAAAATGTATGCGCTATTGAAAAGTGA |
| 83              | wcjC              | Serotype9A, 9V, 9L, 9N  | CGAAAGTGTACCTGAGAATAAGTTTCTTGCAAGATAACAAAAGGCACTTAC |
| 84              | wcjB              | Serotype9A, 9V          | ATTTGAAATTTCGTTGATGATGACTCTCTATTCGCAATTACCCAGAAAGTATGGAATTCCTCTGATGT |
| 85              | wcjB              | Serotype9A, 9V          | TTATCAAATAAAGAGTTTATTAAACCATCTTCTCAAAAATGTATGCGCTATTGAAAAGTGA |
| 86              | wciB              | Serotype10E, 10A, 10B, 10C, 47A | ATCAAGGTAAATCATATCACACTCAGAAATCTTATATTATTGGAATTGGCAAT |
| 87              | wciB              | Serotype10E, 10A, 10B, 10C, 31, 47A | TTAAGATGGAAGCAGCGAGAAATTAAAGAGGTTTTCGCCAGAATATT GCAACCATTTGA |
| 88              | wciB              | Serotype10E, 10A, 10B, 10C, 47A | TGAATTTATTTGGAAGGCAATCCTCTTGTGAAGGCAATTTTGCAAGAAAAATGATGGAAT |
| 89              | wcrC              | Serotype10A, 10C, 34, 35F, 43, 47F, 47A | GTTGCTGTATCTTCTGGCAGAATCTCCTATTATTTGGAATTGATGGAATTCCTCTTGGCAAGAAAAATGATGGAAT |
| 90              | wcrC              | Serotype10A, 10C         | TAACGTGGTGGTCGTTGGCATTATAACAAAAGGATAGTATTATCTTACACGAGTCCGAAAGAA |
| 91              | wcrC              | Serotype10A, 10C         | TGGTTATCTGATAGATTTGTATAGTACCCGATAAGAGTGAGTGAGAAAATTGCTTATTGAGT |
| 92              | wcrD              | Serotype10A, 10B         | GGATATGTTTTCAGGGTTTTTACAGAGTACCCGAAATATTACCTATCTTGGCAGAA |
| 93              | wcrD              | Serotype10A, 10B         | ACCTTATAGAACACCTTCTATCAGTGGCAATTACTCCGTTTATGGAATTGAAAGAAAGAGGATG |
| 94              | wcrD              | Serotype10A, 10B         | TATCGAGAAGATTACAGACAAATTGAGACAGTCTTATGATGATATCTCGAGAAAGAAAGAGGATG |
| 95              | wciF              | Serotype10A, 10B         | AAGCATCATCAGATTGGAATTTTCTTTCTGATACCTGAGATTATTTGGAAGAGAGAATTATAACCTTCAACGAGGATG |
| 96              | wciF              | Serotype10A, 10B         | GGATAAAAATTGTGATTAGTCAGACTCTGCAATAACTATAACTATTACCGTGAAGAGAGAATTATAACCTTCAACGAGGATG |
| 97              | wciF              | Serotype10A, 10B         | AGGCTGCAGCTCCTGGTTTACAGAAATTTCGCAGAATCTTATTGGAATTGAAAGAAAGAGGATG |
| 98              | wcrG              | Serotype10A, 10B         | CTCTGGTGATATTAAAAAGGAACTGATATTATTATTATTGAGGAGCAAGAAGTGAATGTTG |
| 99              | wcrG              | Serotype10A, 10B         | GCTAGAAATATTCAGAAACAAATGTGCTGAAATTTTTGAGCATATTACCCTAGTAAAGGAGAAAAAGGAGAAGG |
| 100             | wcrG              | Serotype10A, 10B         | GCAGCTCAACTGCTGATATTAAAATTCAGATTTTAGTTAAAGGAAACAAATGTGCTGAAATTTTTGAGCATATTACCCTAGTAAAGGAGAAAAAGGAGAAGG |
| 101             | whhK              | Serotype11A, 11B, 11C, 11D, 14, 15F, 15A | GATAGATTAAAAAGTGAGGGGATTATTACGAGGATGTTTTTTATTACGACTGGTTTTTCA |
| Spot identifier | Targeted GT gene | Specificity | Probe sequence (5′–3′) |
|----------------|------------------|------------|----------------------|
| 102            | wchK             | Serotype11A, 11D | TTTTTATGTAATGGAATCCATCATATATATATACACATGGCGG |
| 103            | wchK             | Serotype11F, 11A, 11D | CCGAGGGTTGATATTATCTCCTGAATGTAATAAGCGAATCGTGATATGGTTAGTTAGTTTTG |
| 104            | wcyK             | Serotype11F, 11A, 11D | CATCTAAATATGATATTCTCCTGAATGTAATAAGCGAATCGTGATATGGTTAGTTTTG |
| 105            | wcyK             | Serotype11F, 11A, 11D | CTCGTTGATATTATCTCCTGAATGTAATAAGCGAATCGTGATATGGTTAGTTTTG |
| 106            | wcyK             | Serotype11F, 11A, 11D | GGTCATCATATGTAATGGAATCTTCTGTCATCAATGGATTATATTTGTTAGTTTTG |
| 107            | wcrL             | Serotype11F, 11A, 11D | TGTATGGAAATCAGGAATATTTATGAGATCATAGAGATAAACAGATGCGTTT |
| 108            | wcrL             | Serotype11F, 11A, 11D | GTTATGTCCTGAATTTAAATACAGTCTTGTGTTATACCTTATTCGATCAGT |
| 109            | wcrL             | Serotype11F, 11A, 11D | CAGATCAAAGATATCGTTTATGCTTTCAGCTAAAGAGATCAGT |
| 110            | wciJ             | Serotype12F, 12A, 12B, 44 | GGAATATATAGCTGATTATGGAATCTTCTAATTTGAGACTGATTGAAATGAGGCAAAGAGAGAGGACTATAATAGGTTGTTTC |
| 111            | wciJ             | Serotype12F, 12A, 12B, 44 | ACTTTATTTGGCTGAATTTGGAATCTCAGAAATTTTAGAGACTGATTGAAATGAGGCAAAGAGAGAGGACTATAATAGGTTGTTTC |
| 112            | wciJ             | Serotype12F, 12A, 12B, 44 | AATGGTTGATCAGTTAGTGGAATATTGGAATCTCAGAAATTTTAGAGACTGATTGAAATGAGGCAAAGAGAGAGGACTATAATAGGTTGTTTC |
| 113            | wciJ             | Serotype12F, 12A, 12B, 44 | AAGTTACAATTTGGCTGAATTTGGAATCTCAGAAATTTTAGAGACTGATTGAAATGAGGCAAAGAGAGAGGACTATAATAGGTTGTTTC |
| 114            | wciJ             | Serotype12F, 12A, 12B, 44 | AATGGTTGATCAGTTAGTGGAATATTGGAATCTCAGAAATTTTAGAGACTGATTGAAATGAGGCAAAGAGAGAGGACTATAATAGGTTGTTTC |
| 115            | wciJ             | Serotype12F, 12A, 12B, 44 | AATGGTTGATCAGTTAGTGGAATATTGGAATCTCAGAAATTTTAGAGACTGATTGAAATGAGGCAAAGAGAGAGGACTATAATAGGTTGTTTC |
| 116            | wciJ             | Serotype12F, 12A, 12B, 44 | AATGGTTGATCAGTTAGTGGAATATTGGAATCTCAGAAATTTTAGAGACTGATTGAAATGAGGCAAAGAGAGAGGACTATAATAGGTTGTTTC |
| 117            | wciJ             | Serotype12F, 12A, 12B, 44 | AATGGTTGATCAGTTAGTGGAATATTGGAATCTCAGAAATTTTAGAGACTGATTGAAATGAGGCAAAGAGAGAGGACTATAATAGGTTGTTTC |
| 118            | wciJ             | Serotype12F, 12A, 12B, 44 | AATGGTTGATCAGTTAGTGGAATATTGGAATCTCAGAAATTTTAGAGACTGATTGAAATGAGGCAAAGAGAGAGGACTATAATAGGTTGTTTC |
| 119            | wciJ             | Serotype12F, 12A, 12B, 44 | AATGGTTGATCAGTTAGTGGAATATTGGAATCTCAGAAATTTTAGAGACTGATTGAAATGAGGCAAAGAGAGAGGACTATAATAGGTTGTTTC |
| 120            | wciJ             | Serotype12F, 12A, 12B, 44 | AATGGTTGATCAGTTAGTGGAATATTGGAATCTCAGAAATTTTAGAGACTGATTGAAATGAGGCAAAGAGAGAGGACTATAATAGGTTGTTTC |
| 121            | wciJ             | Serotype12F, 12A, 12B, 44 | AATGGTTGATCAGTTAGTGGAATATTGGAATCTCAGAAATTTTAGAGACTGATTGAAATGAGGCAAAGAGAGAGGACTATAATAGGTTGTTTC |
| 122            | wciJ             | Serotype12F, 12A, 12B, 44 | AATGGTTGATCAGTTAGTGGAATATTGGAATCTCAGAAATTTTAGAGACTGATTGAAATGAGGCAAAGAGAGAGGACTATAATAGGTTGTTTC |
Table 3: Continued.

| Spot identifier | Targeted GT gene | Specificity | Probe sequence (5′–3′) |
|-----------------|------------------|-------------|------------------------|
| 123             | wcxF             | Serotype12F, 12A, 12B, 44, 46 | TGATTTGGTTTTTTGTGGACGTATCAACAAATAAGGATATCAAAAGAATGCCTGGAAGCCT |
| 124             | wcxF             | Serotype12F, 12A, 12B, 44, 46 | GAAATGCTCTCTCGGTATTACTTTCAATAGATTAGAGGAGCCATTTTTTTTTAGTAAGAA |
| 125             | wchK             | Serotype13, 14, 15B, 15C       | TTTATGGGAATATTTTGGATATAGGACTTACTTGTGATATTGATTTTCTGGTCATCAAC |
| 126             | wchK             | Serotype14                       | TAAAAATGCAATTTAGATGATTAGAGGAGCAGGATATTTGCTGGAGCACGATTTAAGGTT |
| 127             | wchK             | Serotype14                       | AGGACAAAATTTTTGAAATTTTGGATATAGGACTTACTTGTGATATTGCAGGATATTTAAGGTT |
| 128             | wchL             | Serotype14, 15B, 15C             | TTGGTGATTAGTGCTTTAAAGCAGAGGAGGATATTTGCTGGAGCACGATTTAAGGTT |
| 129             | wchL             | Serotype14, 15F, 15A             | AAAATTTCTTTGAAATTTTGGATATAGGACTTACTTGTGATATTGCAGGATATTTAAGGTT |
| 130             | wchL             | Serotype14                       | GCTGGTTATTATTTGGATATAGGACTTACTTGTGATATTGCAGGATATTTAAGGTT |
| 131             | wchL             | Serotype14, 15F, 15A, 15B, 15C  | TCCTAAGATTGAGGAGACTCTACTCAAGCAACATATTGAGATTATTGATTTTCTGGAGGAG |
| 132             | wchM             | Serotype14                       | AATAGAAGATTTTTGGAATACAAAGCTGATGATAACCTTGGATTCTATTAGTTCGATGAGG |
| 133             | wchM             | Serotype14                       | AATAGAAGATTTTTGGAATACAAAGCTGATGATAACCTTGGATTCTATTAGTTCGATGAGG |
| 134             | wchM             | Serotype14                       | CAGTAGTTGAATCTGGATTTTGATGTTGATATTGATTTGATTTGATTTGATTTGATTTGATTTG |
| 135             | wchN             | Serotype14, 15F, 15A, 15B, 15C  | CAAAAAATGATATGAACTTTTGGATATTTGAGTTTGGTTCAGGGCGAGAG |
| 136             | wchN             | Serotype14                       | TATGCAGAAAACTACTCTGGGAGTATGGTGAGAAGAAAATAGTTTAGTCAATTATTTT |
| 137             | wchN             | Serotype14                       | GAGTTTTAAATATCAATTTTTCAGAAAAAGGTGGGAAGCACTTAAACCG-ATATCGGGTTT |
| 138             | wchK             | Serotype13, 15B, 15C             | GATGAAAGTTATTATTTCAAGAAATGAGATACCAATTTGCAGAATATTGTTGTGGAGAG |
| 139             | wchK             | Serotype15B, 15C                 | GCTATGGTAATGACCATCAGGTTTTCAGTGTTAAGATGTAAGAATGCAATATTATATCAATATTAT |
| 140             | wchL             | Serotype15B, 15C                 | AGAAAATTTTGAAAGACAGCAATGAGATTTTGGTTGAGTATTGATTTTCTGGAGGAG |
| 141             | wchL             | Serotype15F, 15A, 15B, 15C      | GAAGAAAAATATATACCTTTTTCAGACTGAGATGTCATAGGTGAGATATGTCATTGTGG |
| 142             | wchM             | Serotype15F, 15A, 15B, 15C      | GAAAGATTTTTGGAATACAGACTTATCAAATACTGAGATTTTATTGGTTGTGAGAGGAAAAG |
| 143             | wchM             | Serotype15F, 15A, 15B, 15C      | GCTACGCAATTTGAGTTTACACAAGCAGACTCAGCTAACATAGTGAAGATTTTTAAA-TCTTAATTTT |
| 144             | wchF             |                             | GAAAGCAATTTTGGGGAAATACAGCCCTCAAACAGAGTATGGAAGCTATCCAGAAATTTATG |

| 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 | AAGGTCTTATGGTCAAACATGCAGCTCTTTTAGTGTTAGTAGTAAGA-ATATTGAAAAAT |
| 146             | wchF            | Serotype16F, 17F, 18F, 18B, 18C, 23A | TTCCGTACTTGAAGCATTAGCATCCACAAAGTTAAACTTACTACTCGATGT-TGGTTTTAAA |
| 147             | abp1            | Serotype17F, 24F, 24A, 24B, 48 | GCCAGTCTAATATATCTATACCTTTTACCATCAATAATTTCTATCGCTTGAAAGAATCAGCTAG |
| 148             | abp1            | Serotype17F, 24F, 24A, 24B, 48 | ACAAAACTCCTCCTATTGATTGATAATTTCTATCGCTTGAAAGAATCAGCTAG |
| 149             | abp1            | Serotype17F, 24F, 24A, 24B, 48 | TTAGTTCCTTCTTGGAAAACAGAGAAAAACTGAAAATAGGACTGAAAGAATCAGCTAG |
| 150             | wciP            | Serotype17F | GAAGAAAAGATAAGAGCGAGAAGCGGGAAATATCATGGAGCGCTAT |
| 151             | wciP            | Serotype17F | GTATACACGTCTCATCTATTACACCTTTATGCTCTATAAGTTGTGAGTAAATTACGTTT |
| 152             | wciP            | Serotype17F | ATCTTTAAAGCTATCGGAAATATGAGACTGCTCTGAGAACAAATTCCT |
| 153             | wcrV            | Serotype17F | TCGGAGGATGATGAAACAGAGAATAATGAGTTGAGTAAATTACGTTT |
| 154             | wcrV            | Serotype17F | CATGGAAAAACTTAAAGTTGTTCTGCAAGATATTTAAAATTCAGAGAGCAGTTTATTTAAG |
| 155             | wcrV            | Serotype17F | CGTTTTAATCTACTAAAAATAACGCAGGGAATGTTGGTGACTGCCACTATATATTATTTAAG |
| 156             | wchF            | Serotype17F, 16F, 17F, 18F, 18A, 18B, 18C, 23F, 23A, 24F, 24A, 24B, 28F, 28A, 40, 48 | TATAGGCTATGATATCGCTGCAATTAACAGCTATTGAAAATTCGAA-AGAAATAAGGA |
| 157             | wchF            | Serotype16F, 18F, 18B, 18C | TATAATCAGCTATTAGCAAGTGATTGATAAAAGATCCACGAGTG-AAATTTGTGGA |
| 158             | wciU            | Serotype16F, 18F, 18A, 18B, 18C, 28F, 28A | AGAAAGGATACACCCGACATTACATATACTCAGTTATGGGATTTGCA-TAAAGGAAT |
| 159             | wciU            | Serotype16F, 18F, 18B, 18C, 28F, 28A | TCATCATCAGAGATTGACAATGTGTTGATAAAAGATCCACGAGTG-AAATTTGTGGA |
| 160             | wciU            | Serotype18A, 18B, 18C | GACAAGAGGATTGTTGGCTAATATGACTCAGTTATGGGATTTGCA-TAAAGGAAT |
| 161             | wciV            | Serotype18A, 18B, 18C | AATAATAAATACCTTTATGGAGTATGACTATGTTTATCTATAGATATGCTG-CAAAACTCTA |
| 162             | wciV            | Serotype18F, 18A, 18B, 18C | AGAAGATGCTATTATTTTCAATGTTGTTTTTAAATTAGCAACATCTGCCCT-TGTATTCAC |
| 163             | wciV            | Serotype18F, 18B, 18C | ACCCAATATCCAAATACAGTATTAGCTATTACATCTCGTTATCCACTTTACTAAAC |
| Spot identifier | Targeted GT gene | Specificity | Probe sequence (5′–3′) |
|----------------|------------------|-------------|------------------------|
| 164            | wciW             | Serotype18F, 18A, 18B, 18C | AAGTGCAACTTGAAGATAGGGGCTACACAGAATACTAAAAAGAAATACGGTTCCTTTAAATTT |
| 165            | wciW             | Serotype18F, 18A, 18B, 18C | TGGATTTGACTCAACAGTGTATTITGACAGGAGATTTATTTATTTATTTTGATGAATTTTTTCAGAGGAAAG |
| 166            | wciW             | Serotype18F, 18A, 18B, 18C | TACGACGCGAACCCATATATACTTAGTGGTGAATTTAATAATTTTTTTTTCAGAGGAAAG |
| 167            | wchO             | Serotype19F, 19B, 19C | ATAGATAGTGAGAGAGAATATTTATTTAAGGAGGCTTTGAATAGAGGTTTTTTCAGAGGAAAG |
| 168            | wchO             | Serotype19F, 19B, 19C | GCCTCAAGATATTTAGAGAAGGATTTGATATTGTGGGACAGAGAAATACGG |
| 169            | wchO             | Serotype19F, 19B, 19C | AATTTAGAGGTGTATCTTCAATGAGCCTAAAAACTCCTCTTTTTTTATTTTGATTA |
| 170            | wchO             | Serotype19A | GAGTTCGCGGAATAGCAGGTGAAACATTTACTAGGTGTCATAAGGAAGGAGAATACGG |
| 171            | wchQ             | Serotype19F, 19A, 19B, 19C | ATCGAGATTAGAAAATTTAGGTTTTGATTTAATCTAGGAGAGGCTTTTTTCAGAGGAAAG |
| 172            | wchQ             | Serotype19F, 19A, 19B, 19C | ATCGAGATACGATAGAAAACATTTACTAGGTGTCATAAGGAAGGAGAATACGG |
| 173            | wchQ             | Serotype19F, 19A | GATACGCTTTGTTAAGGCTAAAGGCACCATCAAACATTTCTTTTTTTCAGAGGAAAG |
| 174            | wchQ             | Serotype19F, 19A, 19B, 19C | GCTCAAGATATTTAGAGAAGGATTTGATATTGTGGGACAGAGAAATACGG |
| 175            | wchQ             | Serotype19F, 19A, 19B, 19C | AATTTAGAGGTGTATCTTCAATGAGCCTAAAAACTCCTCTTTTTTTATTTTGATTA |
| 176            | wchQ             | Serotype19F, 19A, 19B, 19C | ATCGAGATACGATAGAAAACATTTACTAGGTGTCATAAGGAAGGAGAATACGG |
| 177            | wchQ             | Serotype19F, 19A | GATACGCTTTGTTAAGGCTAAAGGCACCATCAAACATTTCTTTTTTTCAGAGGAAAG |
| 178            | wchQ             | Serotype19F, 19A, 19B, 19C | ATCGAGATACGATAGAAAACATTTACTAGGTGTCATAAGGAAGGAGAATACGG |
| 179            | wchQ             | Serotype19F, 19A, 19B, 19C | ATCGAGATACGATAGAAAACATTTACTAGGTGTCATAAGGAAGGAGAATACGG |
| 180            | wchQ             | Serotype19F, 19A, 19B, 19C | GATACGCTTTGTTAAGGCTAAAGGCACCATCAAACATTTCTTTTTTTCAGAGGAAAG |
| 181            | wchQ             | Serotype19F, 19A, 19B, 19C | GATACGCTTTGTTAAGGCTAAAGGCACCATCAAACATTTCTTTTTTTCAGAGGAAAG |
| 182            | wchQ             | Serotype19F, 19A, 19B, 19C | ATCGAGATACGATAGAAAACATTTACTAGGTGTCATAAGGAAGGAGAATACGG |
| 183            | wchQ             | Serotype19F, 19A, 19B, 19C | ATCGAGATACGATAGAAAACATTTACTAGGTGTCATAAGGAAGGAGAATACGG |
| 184            | wchQ             | Serotype19F, 19A, 19B, 19C | ATCGAGATACGATAGAAAACATTTACTAGGTGTCATAAGGAAGGAGAATACGG |
| 185            | wchQ             | Serotype19F, 19A, 19B, 19C | ATCGAGATACGATAGAAAACATTTACTAGGTGTCATAAGGAAGGAGAATACGG |
| 186            | wchQ             | Serotype19F, 19A, 19B, 19C | ATCGAGATACGATAGAAAACATTTACTAGGTGTCATAAGGAAGGAGAATACGG |
| 187            | wchQ             | Serotype19F, 19A, 19B, 19C | ATCGAGATACGATAGAAAACATTTACTAGGTGTCATAAGGAAGGAGAATACGG |
| 188            | wchQ             | Serotype19F, 19A, 19B, 19C | ATCGAGATACGATAGAAAACATTTACTAGGTGTCATAAGGAAGGAGAATACGG |
| Spot identifier | Targeted GT gene | Specificity | Probe sequence (5′–3′) |
|----------------|-----------------|-------------|------------------------|
| 189            | *whaF*          | Serotype20  | ACTTTAATACAAAAACTGAAATTTCTCAATTCTATGACTATGTGTTGGCAAGGAGAA |
| 190            | *whaF*          | Serotype20  | ATTTGGTTAGATTCAACGATGATGTTCTCTCAACAAAAGTCAAATTTACTGAGATTGCAGGAAAA |
| 191            | *whaF*          | Serotype20  | AGGGAATATAAAAAAGTACCTCCATATTCTCTGACTAAGACATCTTTTGTTAGGAA |
| 192            | *wchF*          | Serotype22F, 22A | ACTTATATGCTGCCCTATGGAACAGATACAAAGCAGATCTATTTAAAAACCTGATGAGCAAAGAAA |
| 193            | *wchF*          | Serotype18F, 22F, 22A, 23F | ATCTGCTTTTTAGTACGCTCTTGGTTCAACAAAAGGTTAATTTACTGCTGATGTGGCTGGTTTTAAA |
| 194            | *wcwA*          | Serotype22F, 22A | TAAGAAGCAGAGAATGCTGTTTGAGAAGGGGAATAAGATTATTTAGAAGTGG |
| 195            | *wcwV*          | Serotype22F, 22A | GAAAAAGGGGAAAAATAAAGTATTTTGGAAGAGAGAATAAGATTATTTAGAAGTGG |
| 196            | *wcwV*          | Serotype22F, 22A | GGAGAATAAGCAATTTATTTTTATTGAGAAGAGTAAATTTAGAAGTGG |
| 197            | *wcwV*          | Serotype22F, 22A | CCACTTTGAAAAGAGGTTGGAAGCCCTATTTATTTACTGAGATGAGAGGAGTTG |
| 198            | *whaB*          | Serotype22F, 22A | TGGCAATATAGAAGGTTGGAAGCCCTATTTATTTACTGAGATGAGAGGAGTTG |
| 199            | *whaB*          | Serotype22F, 22A | CATCATCAAGATCTTTGTTGGAAGAGTAAATTTACTGAGATGAGAGGAGTTG |
| 200            | *whaB*          | Serotype22F, 22A | TTATTTTACATGGGAGTTGTGTAATTTTTTACACATTATATGTTGTAAGGAGTG |
| 201            | *wchF*          | Serotype23F, 23A | CCACTTTTCTGAGAGGTTGGAAGCCCTATTTATTTACTGAGATGAGAGGAGTTG |
| 202            | *wchV*          | Serotype23F, 23A, 23B | CCTCAATTGGTTGCACTGATGATTGATTTGTCCTCAAGATATGGATATCTTTT |
| 203            | *wchV*          | Serotype23F, 23A | GGCAGATAATTTAAAAAGAGGTTGGAAGAGAGAATAAGATTATTTAGAAGTGG |
| 204            | *wchV*          | Serotype23F, 23A | TTTTGGAGATTACGAAACAATTATTATATTACATTTAGTGGATAATTGTTACGGGTGTAAGCT |
| 205            | *wchW*          | Serotype23F, 23A | ATTTGAAAAAACAAATATTACAAATAACCTTGCCCTACAACAAACTCTTTGCAATGGAAAGGTTG |
| 206            | *wchW*          | Serotype23F, 23A | CGGGGGGATATTATACAAATAAACCTTGCCCTACAACAAACTCTTTGCAATGGAAAGGTTG |
| 207            | *wchW*          | Serotype23F, 23A | CCTATAGCTGAAATACGTCTCCACTACATTAAAAAACAGAGATCCTCAGATGCTCAAGTAAGAT |
| 208            | *wciB*          | Serotype33F, 33A, 34, 35A, 35B, 35C, 37, 41F, 41A, 42 | TTTGTTTATACCGTGCAATAATCTGGCCTAATAATCCTCCTCTCAAATCCATATTATTTGCAGGAT |
| 209            | *wciB*          | Serotype33F, 33A, 34, 35A, 35B, 35C, 37, 41F | ATAGTTCCAAGAGGGAAGTTATTTATGGAACGACTTTTTTACTTTATCTCATTACATTAGCAG |
| 210            | *wciB*          | Serotype33F, 33A, 34, 35A, 35B, 35C, 37, 41F | AACTATTAGATGATTATTACCGTGCAATTATTTATGGAACGACTTTTTTACTTTATCTCATTACATTAGCAG |
| 211            | *wciC*          | Serotype33F, 33A, 37 | CAAATTTTTATATCTGAACAGATGTTTATATTATTTTACTCCAGCTGGTGATGCTGTTGT |
| 212            | *wciC*          | Serotype33F, 33A, 37 | TTACGAAATTATATTGAAGAGTGCTAAAGAAAGATGTTGGGGAAGGATGAAATATCAACATT |
| 213            | *wciC*          | Serotype33F, 33A, 37 | GTTTTACGATGATATTATGAAAGAATTTTACTTTACTCAGATGTTGAGAACAGAAGCCACAT |

Table 3: Continued.
Table 3: Continued.

| Spot identifier | Targeted GT gene | Specificity | Probe sequence (5′–3′) |
|-----------------|------------------|-------------|------------------------|
| 214             | wciD             | Serotype 33F, 33A, 37 | AATAGCAAGAATTTGAGAGAATATGAGGAAATGTAGTTAGTTAT-AAGTAAGGAAAA |
| 215             | wciD             | Serotype 33F, 33A, 37 | TGCAAGAGAACATTTTGGCFTGCTGAAAAATGTTCTATGTAGATA-TGAGATATATTG |
| 216             | wciD             | Serotype 33F, 33A, 37 | GCATAAAACAGTGCTCTATCTATCTTTTAGTTTATATCATCAAGAGTACGCTC-GTAGGTCC |
| 217             | wciE             | Serotype 33F, 33A, 37 | GCAATTTTTAAAAATTTTATACTATCTGGGAGCAAAAGACATGAG-AAGTGGCC |
| 218             | wciE             | Serotype 33F, 33A, 37 | CTGAAATTTTAGAAAAAGATTTTATATCGAGGAGGAAATTTTTC- TAAACCAAGA |
| 219             | wciF             | Serotype 33F, 33A, 37 | TAAGAGTGAGAAATAATTTGAGATGATGATGCTGACGACA-TGAGTGGCC |
| 220             | wciF             | Serotype 33F, 33A, 37 | CTGGGGAAATATTTGAGGAGGAAACTGTGATGATGATGCTGACGACA-TTGTGGCC |
| 221             | wciF             | Serotype 33F, 33A, 37 | CAGAGCTTGTGTGGAATATTTATTTTACTTGAGGAGGAAACTGTGATGATG- TGTGGCC |
| 222             | wciF             | Serotype 33F, 33A, 37 | AAGGAATTTTAGCAGACGCTTTAAATGCTAAAAAGAGTAATTGCTGCTCTTTTAT- TTTGATGAT |
| 223             | 16S              | Streptococcus pneumoniae | TATTTGGAACGAGTACTATAACGCGATAAAGAGTAATTGCTGCTCTTTTAT- TTTGATGAT |
| 224             | 16S              | Streptococcus pneumoniae | ATTAGCTGAGTCCTTAAAGGGCTGCTTTAACATAGTGGCCTTGGGAA- AGAGTTTTAAG |
| 225             | aroE             | Streptococcus pneumoniae | ATTTAAAAACGCTTGTTTCAAAGGTTGTTGATATGATACTGCAGATCA- GAGAGTTTTAAG |
| 226             | aroE             | Streptococcus pneumoniae | AGCAGAGTCTCATTTTACCTGAAATTTGAAAGAAAGGCACACTTTA- AAAACCAAGGAG |
| 227             | aroE             | Streptococcus pneumoniae | TCAGACGCTCATTTCGAGGAGGAAATAGTAATTGCTGCTCTTTTAT- TTTGATGAT |
| 228             | ddl              | Streptococcus pneumoniae | TATTTGGAACGAGTACTATAACGCGATAAAGAGTAATTGCTGCTCTTTTAT- TTTGATGAT |
| 229             | ddl              | Streptococcus pneumoniae | ATTAGCTGAGTCCTTAAAGGGCTGCTTTAACATAGTGGCCTTGGGAA- AGAGTTTTAAG |
| 230             | ddl              | Streptococcus pneumoniae | ATAGCTGAGTCCTTAAAGGGCTGCTTTAACATAGTGGCCTTGGGAA- AGAGTTTTAAG |
| 231             | gdhA             | Streptococcus pneumoniae | TGAATTCTACAAAGCTGCTTTGGAATATTTTACACACTTTTGGAAATAG- GAGAGTTTTAAG |
| 232             | gdhA             | Streptococcus pneumoniae | TAAAGAAAGGATTTTGAATATTTTACACACTTTTGGAAATAG- GAGAGTTTTAAG |
| 233             | gdhA             | Streptococcus pneumoniae | TGAAATTTTTGAAATTTCCTGCAACTGTGATGATGATGCTGACGACA-TGAGTGGCC |
| 234             | gdhA             | Streptococcus pneumoniae | TAAGAGTGAGAAATAATTTGAGATGATGATGCTGACGACA-TGAGTGGCC |
| 235             | gdhA             | Streptococcus pneumoniae | ATTAGCTGAGTCCTTAAAGGGCTGCTTTAACATAGTGGCCTTGGGAA- AGAGTTTTAAG |
| 236             | gcdK             | Streptococcus pneumoniae | AAACAAAGGATTTGGAATATTTTACACACTTTTGGAAATAG- GAGAGTTTTAAG |
| 237             | gcdK             | Streptococcus pneumoniae | AGAATTTCTTACAAAGGATTTGGAATATTTTACACACTTTTGGAAATAG- GAGAGTTTTAAG |
| 238             | spi               | Streptococcus pneumoniae | AGAAGGTATTCTTACAAAGGATTTGGAATATTTTACACACTTTTGGAAATAG- GAGAGTTTTAAG |
| 239             | spi               | Streptococcus pneumoniae | TGATGATGATGCTGATGATGCTGCTTGGAGAAATAGTAATTGCTGACGACA-TGAGTGGCC |
| 240             | spi               | Streptococcus pneumoniae | AACATGCTGCTTTTACACACTTTTGGAAATAGTAATTGCTGACGACA-TGAGTGGCC |
| 241             | tktA             | Streptococcus pneumoniae | ACAATTTTTTACACACTTTTGGAAATAGTAATTGCTGACGACA-TGAGTGGCC |
| 242             | tktA             | Streptococcus pneumoniae | ACAATTTTTTACACACTTTTGGAAATAGTAATTGCTGACGACA-TGAGTGGCC |
| 243             | tktA             | Streptococcus pneumoniae | ACAATTTTTTACACACTTTTGGAAATAGTAATTGCTGACGACA-TGAGTGGCC |
| 244             | tktA             | Streptococcus pneumoniae | ACAATTTTTTACACACTTTTGGAAATAGTAATTGCTGACGACA-TGAGTGGCC |
| 245             | tktA             | Streptococcus pneumoniae | ACAATTTTTTACACACTTTTGGAAATAGTAATTGCTGACGACA-TGAGTGGCC |
| 246             | tktA             | Streptococcus pneumoniae | ACAATTTTTTACACACTTTTGGAAATAGTAATTGCTGACGACA-TGAGTGGCC |
| 247             | tktA             | Streptococcus pneumoniae | ACAATTTTTTACACACTTTTGGAAATAGTAATTGCTGACGACA-TGAGTGGCC |
| 248             | tktA             | Streptococcus pneumoniae | ACAATTTTTTACACACTTTTGGAAATAGTAATTGCTGACGACA-TGAGTGGCC |
| 249             | tktA             | Streptococcus pneumoniae | ACAATTTTTTACACACTTTTGGAAATAGTAATTGCTGACGACA-TGAGTGGCC |
| 250             | tktA             | Streptococcus pneumoniae | ACAATTTTTTACACACTTTTGGAAATAGTAATTGCTGACGACA-TGAGTGGCC |
| Spot identifier | Targeted GT gene | Specificity                  | Probe sequence (5′–3′) |
|-----------------|------------------|-----------------------------|------------------------|
| 242             | tktA             | Streptococcus pneumoniae    | CAATCAAGATGCTATCAAAGTGCATTTTCATATACAAACATAGTGACCTTACCCAGGA |
| 243             | tktA             | Streptococcus pneumoniae    | TTCAAGATTTGGTCCCATGGAATTCAGCAGAAACAAAATGATGTGACCATTAACCCAGGA |
| 244             | tktA             | Streptococcus pneumoniae    | AACATGTTCTTTGAAATCAGCATATACTTGTTCTGGAATTTCAATATGCTTCGTTTCA |
| 245             | tktA             | Streptococcus pneumoniae    | TCACCAGATAGAATGATAGTGTAGTTGCTCCATCATATAGTTGATGAGGAGATGTTGAT |
| 246             | xpt              | Streptococcus pneumoniae    | AGATTTCCCTTTAACCACCAAGTGACCTTATGATGGAGGAGGCTTACCATATAGTTGAT |
| 247             | xpt              | Streptococcus pneumoniae    | ATGATTTTGGCCAAAAGACTAAGAATATCACCAGAAGGACATTCGATGTGCATACCCATG |
| 248             | xpt              | Streptococcus pneumoniae    | TTTGATTTATCGAGGATTTGCTATTGCTAATGGAACGTGTAAGGAGGCTTACCATATAGTTGAT |
| 249             | KP<sub>gapA</sub> | Klebsiella pneumoniae       | GAGCAGTGTTCTGGCAAGAAGAGCCAATGGCTATTCTCCAGGCACAGGACATTCGAT |
| 250             | KP<sub>rpoB</sub> | Klebsiella pneumoniae       | AAGGCGTTGTGTTACTGGAAGGAGCACTGAAACATTCCAGGCAATTCGATCTTTGAT |
| 251             | KP<sub>mdh</sub> | Klebsiella pneumoniae       | GTAGATGGTATTTGGAAGGCAATTCGAAACATTCCAGGCAATTCGATCTTTGAT |
| 252             | KP<sub>pgi</sub> | Klebsiella pneumoniae       | TCTCGCCCTTTGCTCTAATATCCAGGAAAAATACAGGATGAGTTAATGGAACATTCGAT |
| 253             | SA<sub>arcC</sub> | Staphylococcus aureus       | TGATAGGCTATTTGGAAGAATATACTCCGACATGATTCGATCTTTGAT |
| 254             | SA<sub>aroE</sub> | Staphylococcus aureus       | AAGTTTTTGATTTGGCATTAGTCTCTGGTATATTTGATGGCTTAAAATGTTAGG |
| 255             | SA<sub>glpF</sub> | Staphylococcus aureus       | TAAAGATTACTTTGGCCAAAATACAGGATGAGTTAATGGAACATTCGATCTTTGAT |
| 256             | SA<sub>gmk</sub> | Staphylococcus aureus       | CGTAAAGGGGTTTGGATATTATGTAATATTTGATGGCTGAAATACAGGATGAGTTAATGGAACATTCGATCTTTGAT |
| 257             | LP<sub>acnF</sub> | Legionella pneumophila      | CCAAAAGGAGGGTTTGGATATTATGTAATATTTGATGGCTGAAATACAGGATGAGTTAATGGAACATTCGATCTTTGAT |
| 258             | LP<sub>mompS</sub> | Legionella pneumophila      | TCAATGIGAACTGGATTTGATTTATGTAATATTTGATGGCTGAAATACAGGATGAGTTAATGGAACATTCGATCTTTGAT |
| 259             | CP<sub>groES</sub> | Chlamydia pneumoniae        | TTTCTTACCTGCTGATTTGGAATATATGGAACATGCTTTTGTGTTGTAATATTTTGTGTTG |
| 260             | CP<sub>gyrA</sub> | Chlamydia pneumoniae        | GTTTGCTGGCTTAATAATAGAAGGAGGAGGCAATGATATATGGAACATGCTTTTGTGTTG |
| 261             | CP<sub>gyrB</sub> | Chlamydia pneumoniae        | CCAAGACCTTTTACACCCTGATAATATGGAACATGCTTTTGTGTTGTAATATTTTGTGTTG |
| 262             | CP<sub>hcaA</sub> | Chlamydia pneumoniae        | CTCGCTGCTCTAATAATAATGGAACATGCTTTTGTGTTGTAATATTTTGTGTTG |
| 263             | CP<sub>accA</sub> | Chlamydia pneumoniae        | TGATATAGGTAATACATTGCAATGAAATATGGAACATGCTTTTGTGTTGTAATATTTTGTGTTG |
| 264             | CP<sub>hcaK</sub> | Chlamydia pneumoniae        | TCAAAAACGAGAGGCAATTGCTATCCAATCCAGAGATATATGGAACATGCTTTTGTGTTG |
| 265             | MP<sub>gyrB</sub> | Mycoplasma pneumoniae       | AGGAAACCTTTATTGAGGAGCATTATAATGGAACATGCTTTTGTGTTGTAATATTTTGTGTTG |
| 266             | MP<sub>gyrA</sub> | Mycoplasma pneumoniae       | ACAAGATCAAATGGAATATATGGAACATGCTTTTGTGTTGTAATATTTTGTGTTG |
| 267             | MP<sub>hcm</sub> | Mycoplasma pneumoniae       | TTTGCCGAAGCTCAAGAATTTATACCTAATCCAGAGAATATATGGAACATGCTTTTGTGTTG |
Table 3: Continued.

| Spot identifier | Targeted GT gene | Specificity | Probe sequence (5′–3′) |
|-----------------|------------------|-------------|------------------------|
| 268             | MP_{gt}          | Mycoplasma pneumoniae | TGGGATTGCGCTTTGGCATCTTAATGTTTGTCTTGAGTTAATTTACTTTTACAGATTCA |
| 269             | MP_{fus}         | Mycoplasma pneumoniae | TAAGCTCCGGTAAACCTCCTCAAAAGAGAGTTGAGGTTGAAATTACATTAAACAAAT |
| 270             | MP_{ispA}        | Mycoplasma pneumoniae | TTTTGGAAAAATGGTGATCGAACGTTACTCCAACGTCATGCACATCGGGTCTCCACTGTCGA |
| 271             | PA_{trpE}        | Pseudomonas aeruginosa | TACCAGAAAAATGGTGATCGAAGCTTACCCCAAGGTCACTGCACATCGTGTTCCACCTATAT |
| 272             | PA_{nuoD}        | Pseudomonas aeruginosa | GATCATGTGCGGAGGTTCCTCCGTATCTCGAAGCCACCTGCTATCCGTTCCACCTATAT |
| 273             | SP_{gki}         | Streptococcus pyogenes | ATTCAGCCATGAAAAGCAGCTATTTGAATGGTGAAAGTGTTACCAGTAAGACATTTCATA |
| 274             | SP_{xpt}         | Streptococcus pyogenes | ATGGCCTGTTAATTTCTTATCTAAAGAAGACAAGTTTTGATTATTGATGACCTTTTAGCT |

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.

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
Serotype 3 (strain ID: D36)

Serotype 11A (strain ID: SSI 11A/2)

Serotype 9V (strain ID: KD10-11)

Strain 19F (strain ID: D33)

Serotype 22F (strain ID: KD01–23)

Strain 22A (strain ID: ATCC10363)

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                        | Assigned group |
|----------|---------------|----------------------------------------------------------------------------------|------------------------------------------|----------------|
| 1        | ATCC6301      | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18                      | Perfectly matched                         | 1              |
| 2        | ATCC6302      | 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       | 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, 59, 60, 61, 62, 63    | Perfectly matched                         | 7F/7A          |
| 8        | ATCC6308      | 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, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137 | 1 extra probe of group 2 hybridized 12F/12A/12B/44/46 | |
| 14       | D59           | 125, 128, 131, 135, 138, 139, 140, 141, 142, 143                               | Perfectly matched                         | 14             |
| 15B      | ATCC10354     | 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156                | 1 extra probe of group 18B/18C hybridized | 17F            |
| 17F      | ATCC6317      | 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166                           | Perfectly matched                         | 18B/18C        |
| 18C      | ATCC10356     | 167, 168, 169, 171, 172, 173                                                    | Perfectly matched                         | 19F            |
| 19F      | D33           | 71, 73, 74, 169, 170, 171, 172, 173                                             | 1 extra probe of group 19F hybridized    | 19A            |

23 serotypes included in 23-valent vaccine
| Serotype | Strain ID  | Positive probea | Microarray result | Assigned 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, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222 | 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     |
| 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, 247 | Perfectly matched | 12F/12A/12B/44/46 |
| 12B      | SSI12B/1 | 125, 128, 131, 135, 138, 139, 140, 141, 142, 143 | Perfectly matched | 12F/12A/12B/44/46 |
| 15C      | SSI15C/2 | 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 7, 48, 49, 192, 193, 194, 195, 196, 197, 198, 199, 200 | Perfectly matched | 15B/15C        |
| 18B      | ATCC10355 | 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222 | Perfectly matched | 18B/18C        |
| 22A      | ATCC10363 | 22F/22A | Perfectly matched | 22F/22A        |
| 33A      | ATCC8340 | 33F/33A/37 | Perfectly matched | 33F/33A/37     |
Table 4: Continued.

| Serotype | Strain ID     | Positive probe<sup>a</sup> | Microarray result                        | Assigned 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: <sup>a</sup>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.

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