

Supplemental appendix 1: Details of PCR detection method for each virulence factor gene.

Conventional PCR was carried out on Techne TC-512 thermocyclers (Fisher Bioblock Scientific), using a standard 25 µL mix containing 2.5 µL DNA solution + 5 µL Green GoTaq® reaction buffer (5X) + 200 µM (each) deoxynucleoside triphosphate + 0.4 µM (each) primers + 1 unit of GoTaq® G2 DNA polymerase (Promega). For reaction involving high PM products (PspC detection), expand high fidelity PCR system (Roche) was used as previously published (Ianello F et al. Gene 2002). Real-time PCR was performed on Stratagene Mx3005P instrument (Agilent Technologies) using a 25µL mix containing 5µL DNA suspension + 5µL reaction mix (5X) LC FastStart DNA MaterPlus Hybprobe (Roche) + 0.9 µM (each) primers + 0.25 µM probes. For each gene, primers and probes sequences as well as PCR amplification conditions are detailed in the table below.

Virulence factors genes	Name	Primers and Probes		Conditions of amplifications			Positive control (reference strain)	References
		Name	Sequences (5'→3')	initial denaturation	N cycles [(denaturation), (hybridation), (elongation)]	final extension		
<i>Conventional PCR</i>								
<i>pspA</i>	all types	primer-F (LSM12)	CCGGATCCAGCGTCGCTATCTTAGGGGCTGGTT	95 °C (3 min)	30 [95 °C (1 min), 62 °C (1 min), 72 °C (3 min)]	72 °C (10 min)	R6 / Tigr4	Hollingshead S <i>et al.</i> J Med Microbiol 2006; 55:215-21
		primer-R (SKH02)	CCACATACCGTTTTCTTGTCCAGCC					
	Family 1	primer-F (LSM12)	CCGGATCCAGCGTCGCTATCTTAGGGGCTGGTT	95 °C (3 min)	30 [95 °C (1 min), 62 °C (1 min), 72 °C (3 min)]	72 °C (10 min)	R6	Hollingshead S <i>et al.</i> J Med Microbiol 2006; 55:215-21
		primer-R (SKH63)	TTTCTGGCTCATYAACTGCTTTC					
Family 2	primer-F (LSM12)	CCGGATCCAGCGTCGCTATCTTAGGGGCTGGTT	95 °C (3 min)	30 [95 °C (1 min), 62 °C (1 min), 72 °C (3 min)]	72 °C (10 min)	Tigr4	Hollingshead S <i>et al.</i> J Med Microbiol 2006; 55:215-21	
	primer-R (SKH52)	TGGGGGTGGAGTTTCTTCTCATCT						
Family 3	primer-F (SKH41)	CGCACAGACTAACAGATGAAC	95 °C (3 min)	30 [95 °C (1 min), 62 °C (1 min), 72 °C (3 min)]	72 °C (10 min)	NA	Hollingshead S <i>et al.</i> J Med Microbiol 2006; 55:215-21	
	primer-R (SKH42)	CTTGTCATCAACTTCATCC						
<i>pspC</i>	all types	primer-F (IF30)	AAGATGAAGATCGCTACGAACAC	94 °C (4 min)	30 [92 °C (30 sec), 50 °C (30 sec), 68 °C (10 min)]	68 °C (20 min)	R6 / Tigr4	Ianello F <i>et al.</i> Gene 2002; 284:63-71
		primer-R (IF43)	AATGAGAAACGAATCCTTAGCAAT					
	Group 4	primer-F (LU9)	GAAGAGTTAGAAGAGGGAATAACCTCACGGT	96 °C (5 min)	30 [96 °C (45 sec), 50 °C (45 sec), 72 °C (3 min)]	72 °C (10 min)	NA	Dieudonne-Vatran A <i>et al.</i> J Immunol 2009; 182: 7865–77
		primer-R (LU10)	GTTTACCCATTTACCATTGGCATTGACTCTA					
<i>ply</i>		primer-F (JVS59L)	TGAGACTAAGTTACAGCTTACAG	95 °C (5 min)	35 [95 °C (20 sec), 55 °C (30 sec), 68 °C (1 min)]	68 °C (10 min)	R6 / Tigr4	Sakai F <i>et al.</i> Plos One 2013; 8:e67147
		primer-R (JVS60R)	CTAATTTTACAGAGAGATTACGA					
		primer-Fbis ⁸ (new)	ATGGAAATCGCTAGGCAAGA	95 °C (5 min)	30 [95 °C (45 sec), 55 °C (45 sec), 72 °C (90 sec)]	728 °C (5 min)	R6 / Tigr4	Home made
		primer-Rbis ⁸ (new)	TTAGTCCAACCCAGCGCTGAT					
<i>pavA</i>		primer-F (JVS65L)	CGATAAAAGCAGTCATAAAATCCT	95 °C (5 min)	35 [95 °C (20 sec), 55 °C (30 sec), 68 °C (1 min)]	68 °C (10 min)	R6 / Tigr4	Sakai F <i>et al.</i> Plos One 2013; 8:e67147
		primer-R (JVS66R)	AGGATTGAGAGATTCTGTACTTGG					
<i>lytA</i>		primer-F (JVS1L)	AGTTAAGCATGATATTGAGAAC	95 °C (5 min)	35 [95 °C (20 sec), 55 °C (30 sec), 68 °C (1 min)]	68 °C (10 min)	R6 / Tigr4	Sakai F <i>et al.</i> Plos One 2013; 8:e67147
		primer-R (JVS2R)	TTCGTTGAAATAGTACCATTAT					
<i>phtA</i>		primer-F	TTCTTACGAGTTGGGACTGTATCAAGC	96 °C (5 min)	30 [96 °C (45 sec), 54 °C (45 sec), 72 °C (3 min)]	72 °C (10 min)	R6 / Tigr4	Adamou J.E. <i>et al.</i> Infect Immun 2001; 69: 949-58
		primer-R	GTTTATTTTTCTTACTTACAGATGAAGG					
<i>phtB</i>		primer-F	TGCCCTAAGTGTTCCTATGAGCT	96 °C (5 min)	30 [96 °C (45 sec), 54 °C (45 sec), 72 °C (3 min)]	72 °C (10 min)	Tigr4	Adamou J.E. <i>et al.</i> Infect Immun 2001; 69: 949-58
		primer-R	TTACTTACTCTCCTTAATAAAGCCAATAG					
<i>phtD</i>		primer-F	TCCTATGAACCTGGTCGTC	96 °C (5 min)	30 [96 °C (45 sec), 54 °C (45 sec), 72 °C (3 min)]	72 °C (10 min)	R6 / Tigr4	Adamou J.E. <i>et al.</i> Infect Immun 2001; 69: 949-58
		primer-R	TTACTATATAGGAGCCGGTTGACT					
		primer-Rbis* (PhtD-R new)	TTTCGTTCCGAGAAGAAGACT					Home made
<i>phtE</i>		primer-F	GCCTATGCACTAAACCAGCA	96 °C (5 min)	30 [96 °C (45 sec), 54 °C (45 sec), 72 °C (3 min)]	72 °C (10 min)	R6 / Tigr4	Adamou J.E. <i>et al.</i> Infect Immun 2001; 69: 949-58
		primer-R	CTAAATGTTTTGCGCAGCT					
Pilus islet-2	presence	primer-F (sipA-up)	CGTGGGTATCAGGTGTCCTATGATAA	95 °C (15 min)	35 [95 °C (20 sec), 55 °C (30 sec), 68 °C (1 min)]	68 °C (10 min)	NA	Aguir SI <i>et al.</i> Vaccine 2012; 30:5487-90
	absence	primer-R (sipA-dn)	GCCTCGTCTTCTAATGACTGTTAC					
		primer-F (1008for)	GCTGGATCGAGTTTAAACCAGAA	95 °C (15 min)	35 [95 °C (20 sec), 55 °C (30 sec), 68 °C (1 min)]	68 °C (10 min)	R6 / Tigr4	Bagnoli F <i>et al.</i> J of Bacteriology 2008; 190:384-08
		primer-R (1009rev)	TAAGGATCACCAAAGTCCAAGGCA					
<i>nanA</i>		primer-F	ATAGACGTGCGCAAAATACAGAATCA	98 °C (5min)	35 [98 °C (30 sec), 55 °C (30 sec), 72 °C (1 min)]	72 °C (10 min)	R6 / Tigr4	Pettigrew M <i>et al.</i> Infect Immun 2006; 74:3360-65
		primer-R	GTCGAACTCCAAGCCAATAACTCCT					
<i>nanB</i>		primer-F	ACTACGAGGTGTTAATCGTGAAGG	98 °C (5min)	35 [98 °C (30 sec), 55 °C (30 sec), 72 °C (1 min)]	72 °C (10 min)	R6 / Tigr4	Pettigrew M <i>et al.</i> Infect Immun 2006; 74:3360-65
		primer-R	CCAATACCCGCGCAGGCATAACATC					
<i>nanC</i>		primer-F	TGGGGTAAGTACAACAAGAGG	98 °C (5min)	35 [98 °C (30 sec), 51 °C (30 sec), 72 °C (1 min)]	72 °C (10 min)	Tigr4	Pettigrew M <i>et al.</i> Infect Immun 2006; 74:3360-65
		primer-R	CTAATGGTACTGGCGCAAAATCA					
<i>pcpA</i>		primer-F (SP2136F)	CCCCTCACCCTTAGCATT	96 °C (5 min)	30 [96 °C (45 sec), 54 °C (45 sec), 72 °C (3 min)]	72 °C (10 min)	R6 / Tigr4	Browall S <i>et al.</i> J Infect Dis 2014; 209:377-88
		primer-R (SP2136R)	CCTAGTTCGGAAGTAATCAA					
<i>Real-time PCR</i>								
Pilus-1	<i>rrgA</i>	primer-F	TTGTGACAAATCTTCTCTTGGGA	95 °C (10min)	45 [95 °C (15 sec), 60 °C (60 sec)]		Tigr4	Selva L <i>et al.</i> PLoS ONE 2012; 7: e41587
		primer-R	GTCACCAGCTGATGATCTACCA					
		probe	FAM-CAGTGGCTCCACCTCC (Quencher MGB)					
<i>psrp</i>		primer-F	CTTTACATTTACCCCTTACGCTGCTA	95 °C (10min)	45 [95 °C (15 sec), 60 °C (60 sec)]		Tigr4	Selva L <i>et al.</i> PLoS ONE 2012; 7: e41587
		primer-R	CTGAGAGTGACTTAGACTGTGAAAGTG					
		probe	FAM-CTGGTCTGCTAGATTC (Quencher MGB)					

Table Legend: ⁸second pair of primers designed by alignment with *ply* gene sequence from the R6 reference strain *new primer-R designed by alignment of *phtD* gene sequence from the three reference strains R6 (accession number NC_003098.1), D39 (accession number NC_008533.1), ATCC700669 (accession number NC_011900.1) using ClustalW program. Abbreviations: F=forward, R=reverse, N=number, min=minutes, sec=seconds, NA=not available, ply=pneumolysin, pspA=pneumococcal surface protein A, pspC=pneumococcal surface protein C, pavA=pneumococcal adhesion and virulence A, lytA=autolysin A, pht=polyhistidine triad complex, nan=neuraminidase, pcpA=pneumococcal choline binding protein A, psrp=pneumococcal serine-rich protein.