Supplementary tables and figures

Table S1. Oligonucleotide primers and probe sequences for the 'Fluidigm' qPCR reaction-set (96 assay-sets) for detection of 94 pneumococcal serotypes, 15 other bacterial and one fungal species

Assay-set name	*Forward primer (5′ to 3′)*	*Reverse primer (5′ to 3′)*	*Probe (5′ to 3′)*	*Gene Target*	*Reference*
S. pneumoniae Serotype 1 †	CGTGCGGTAATTGAAGCTATGA	TGTGGCCCCAGCAACTCT	FAM-TGCTTGCCCTTGTATAGGGT-NFQ	wch*D*	Azzari *et al.* [1]
S. pneumoniae Serotype 2 ‡	TTATGGACTGGCTGATGGTTCTC	AAATCCTGACCCAATAATAGCCTTT	FAM-AGGTCAACG/ZEN/TATTGGAACTCTTAGAAATTGGGAAA-IABkFQ	wzy	Pholwat *et al.* [2]
S. pneumoniae Serotype 3 †	GGTCAGCAGAAAGTATGCATTGG	TCGTTTATCCAGGGTCTGATGA	VIC-TATTGGATGTGGTTTATCGTGAAGA-NFQ	tnp	Azzari *et al.* [1]
S. pneumoniae Serotype 4 †	TGGGATGACATTTCTACGCACTA	CCGTCGCTGATGCTTTATCA	FAM-TCCTATTGGATGGTTAGTTGGTGA-NFQ	wzy	Azzari *et al.* [1]
S. pneumoniae Serotype 5 †	TTACGGGAGTATCTTATGTCTTTAATGG	CAGCATTCCAGTAGCCTAAAACTAGA	VIC-TTGTCTCAGCAACTCTATTTGGCTGTGGG-NFQ	wzy	Azzari *et al.* [1]
S. pneumoniae Serogroup 6A/B/C/D (F/G/H) †	AAGTTTGCACTAGAGTATGGGAAGGT	ACATTATGTCCRTGTCTTCGATACAAG	VIC-GTTCTGCCCTGAGCAACTGG-NFQ	wci*P*	Azzari *et al.* [1]
S. pneumoniae Serotype 6A/C ‡	CATTGCTAGAGATGGTTCCTTC AGTTGATATTGATAAAGATTCG GGAGACATGTCCAAACTGGC	CGATACAAGACCAGTTGC	FAM-GTTTGCACT/ZEN/AGAGTATGGGAAGGTGTTGT-IABkFQ	wci*P*α	Downs *et al.* [3]
S. pneumoniae Serotype 6C/D †	TTGGGATGATTGGTCGTATTAG	CTCTTCAATTAGTTCTTCAGTTCG	FAM-CCACGCAATTCGCCATC-NFQ	wciN_{β}	Azzari *et al.* [1]
S. pneumoniae Serogroup 7A/F †	GATGGCATGTGGCAAACCA	TTTGCCCTCCTTAATCATTTCAC	FAM-TTGGCTATCGGCATGGTGGT-NFQ	wcw*H*	Azzari *et al.* [1]
S. pneumoniae Serogroup 7B/C/40 ‡	TCCAGATATAGTCATTCCCAATCAG	AAAGAAGGTAAATCCCATGATGAATT	FAM-TCCCTCATTATCGATTACTGACCCACCA-BHQ1	wcx*U*	Pholwat *et al.* [2]
S. pneumoniae Serotype 8 †	CCACTCATCAGTTTCCCATATGTTT	TCAATAATTGAAGAAGCGAACGTT	FAM-TGATGGCAGATGGGTTGGGACGAG-NFQ	wzx	Azzari *et al.* [1]
S. pneumoniae Serogroup 9A/L/N/V ‡	TGGAATGGGCAAAGGGTAGTA	TCGGTTCCCCAAGATTTTCTC	FAM-TTAATCATGCTAACGGCTCATCGA-BHQ1	mna*A*	Olwagen *et al.* [4]
S. pneumoniae Serogroup 9A/V‡	AGGTATCCTATATACTGCTTTAGG	CGAATCTGCCAATATCTGAAAG	FAM-ACACATTGACAACCGCTACA-BHQ1	wzx	Pholwat *et al.* [2]
S. pneumoniae Serogroup 9L/N ‡	CGTGGAATTTTCTATACTGCAATAGG	CTACTGCTACGATACCATATTCTACAG	FAM-CAATTCTTAGCCGGATTCTCTC-BHQ1	wzx	Pholwat *et al.* [2]
S. pneumoniae Serogroup 10A/B †	CCTCTCCTATCAACTATTACTCATTATACTACCT	AATAACCATAAGTCCCTAGATCATTCAAAG	VIC-TCATTACAACTCCCTATGTGACACGGGTCTTTT-NFQ	wcr*D*	Azzari *et al.* [1]
S. pneumoniae Serotype 10B †	AAATATGAGATTGGTAAGGAATATTCTGG	GTCTTTTCACTTAAACGAATTCCATTC	FAM-AACGGATTCCAATGCACTCGGTAACT-NFQ	wcr*D*	Pholwat *et al.* [2]

S. pneumoniae Serogroup 10C/F †	CGAGTTATGGATGTTCTTATTGGC	CCCAACCCCACTCTGTATTG	FAM-ACAGGGCAAGACTGTGAATATTGTTCCA-NFQ	wcj*G*	Sakai *et al.* [5]
S. pneumoniae Serogroup 11A/B/C/D/F/(E) †	ACCGCATTTCTTATCGCACTATATT	TCTCCTTACCATCAAACATGTTAATCA	FAM-TGAATCAGTCTGACCGTTT-NFQ	wzx	Olwagen *et al.* [4]
S. pneumoniae Serogroup 11A/D †	CGGCCCAGCTACATTTATGG	TGATCATTCACATGCTCACCAA	FAM-AAATACCAATAGTTGTTCCGAGATTAAAGAAGT-NFQ	wch*K*	Pholwat *et al.* [2]
S. pneumoniae Serogroup 11B/C †	TCAAATTTGGCGTATTGCTTATCA	TGATTATGAGCATAGTTGATCCCC	FAM-TCCGTGGCAAGATTCTGGTGCTAAG-NFQ	wzy	Sakai *et al.* [5]
S. pneumoniae Serotype 11F †	TGGTCCAGCTACTTTTATGGC	TGATCATTCACATGCTCCCC	VIC-ACTCCAATAGTTGTTCCGAGGCAAAAGA-NFQ	wch*K*	Pholwat *et al.* [2]
S. pneumoniae Serogroup 12A/B/F/44/46 †	GATTATTCGCTTGCCTCTTCATG	ATAGCCGAAATAAGCTTTCCAGAA	FAM-ATTTGTAAGCGGACGTGCGATT-NFQ	mna*B*	Azzari *et al.* [1]
S. pneumoniae Serotype 12B †	GGTTGCTGATCAAAAGGTCTATG	AGGTTCAAAGTAAGATTTTTAGCAA	FAM-AGATAAAAATCTTTCCAAATCATCAAAGTGA-NFQ	wzx	Pholwat *et al.* [2]
S. pneumoniae Serotype 13 †	TCGGATTTAGTAGTAACCCCATTGA	TTCTTGATTGAGGATGCATTTCC	VIC-AGTAGTAAGAGATCATATTCAAG-NFQ	wzy	Olwagen *et al.* [4]
S. pneumoniae Serotype 14 †	CGACTGAAATGTCACTAGGAGAAGAT	AATACAGTCCATCAATTACTGCAATACTC	VIC-TGTCATTCGTTTGCCAATACTTGATGGTCTC-NFQ	wch*L*	Azzari *et al.* [1]
S. pneumoniae Serogroup 15A/B/C/F ‡	TTGAATCAGGTAGATTGATTTCTGCTA	CTCTAGGAATCAAATACTGAGTCCTAATGA	FAM-CTCCGGCTTTTGTCTTCTCTGT-BHQ1	wzx	Azzari *et al.* [1]
S. pneumoniae Serogroup 15A/F ‡	CGTTATTTAGTGAATTGCTATACTC	TCCCTGCAGAATAAGAATCTAC	FAM-TACtGCtGCtGCcAACA-BHQ1	wzy	Messaoudi *et al.* [6]
S. pneumoniae Serogroup 15B/C ‡	TTTGCTACAGGTTTTAGTATTGAG	AAAGCAATATAAGAGGTATAGTTGG	FAM-CGCtACaATcATcCGCT-BHQ1	wzy	Messaoudi *et al.* [6]
S. pneumoniae Serotype 16A †	GCTAGCAGGAACTTTTCTAGGG	TCCCTGTCCAAATCCGAAACCATTATGGCG-NFQ	FAM-CCCACGGGATGAATC	wcx*R*	Sakai *et al.* [5]
S. pneumoniae Serotype 16F †	GCAACTGGTATTTTTGATATTGGAGAA	CAAAGGAATGCCATGCCATATTCGTTGGAGG-NFQ	FAM-AAAATGCTAAC	wzy	Olwagen *et al.* [4]
S. pneumoniae Serotype 17A †	TGATTATGTCATTCGATTGCTTGG	AAATCCTAAAATTCCTGTTTGAAAAGC	FAM-ATTATGGGCGTGGGTTACCGTAGG-NFQ	wzy	Sakai *et al.* [5]
S. pneumoniae Serotype 17F †	GTAAAGATTTCATGTCCTATAAGGGAGAA	AGGCGTCCCTGTTTATGAGAAG	FAM-TTGTACATGGTCTGGATTT-NFQ	Wzx	Olwagen *et al.* [4]
S. pneumoniae Serogroup 18A/B/C/F ‡	CCTGTTGTTATTCACGCCTTACG	TTGCACTTCTCGAATAGCCTTACTC	FAM-AACCGTTGGCCCTTGTGGTGGA-BHQ1	wzy	Azzari *et al.* [1]
S. pneumoniae Serogroup 18B/C/F ‡	CAGGATTTCTAACTCTGATTGAA	AGCAAAATCTAACGTCCAGAG	FAM-CTTGTATGCTTATGGTCTTTTCGATTA-BHQ1	wciX_{BCF}	Downs *et al.* [3]
S. pneumoniae Serogroup 18C/F ‡	CCAAATTGGAGTGTTTTACAAAGTATTAGCTCGATTTGCTGTACACGTCGACGCTTCAATTTCAGG	TCTTTCAAATACAACTCTTAGATTTCCTTGTG	FAM-TGagtTTATTGATAATttcC-BHQ1	wciX_{CF}	Downs *et al.* [3]

S. pneumoniae Serotype 18F:	TGGTTTCGGACTCTTTCGTGG	CTAAGATAGAAACTCCTTGTCCAATG	FAM-GGTTGTACGTGGAATCGGATTTGGTC-BHQ1	wcx*M*	Downs *et al.* [3]
S. pneumoniae Serogroup 16F/18F/28AF ‡					
S. pneumoniae Serotype 19A †	TTCGACGACGTATCAGCTTCA	TCATTGAGAGCCTTAACCTCTTCA	VIC-ACCCAAAACGGTTGACGCATTATACT-NFQ	wzy	Azzari *et al.* [1]
S. pneumoniae Serogroup 19B/F †	GGTCATGCGAGATACGACAGAA	TCCTCATCAGTCCCAACCAATT	VIC-ACCTGAAGGAGTAGCTGCTGGAACGTTG-NFQ	wzy	Azzari *et al.* [1]
S. pneumoniae Serotype 19F †	CAGGTTCGGGAAATTGCAA	ATCTCTGCGCCATAAGCAATG	VIC-AGAAGTGGCAGATGATT-NFQ	wzh	Downs *et al.* [7]
S. pneumoniae Serotype 20 †	AAAGATACTGGCTGAGGAGCTATCTATT	AGTCAAAAGTACTCAACCATTCTGATATATTC	VIC-AGGATAAGGTCTACTTTGTGGGAGTTC-NFQ	wci*L*	Azzari *et al.* [1]
S. pneumoniae Serotype 21 †	CCATTTGAAGGACCAGTTGTTG	AAAAAGCCACTATCAGGAATACCAA	FAM-AATGGCATTGCTTCGTAAA-NFQ	wzy	Olwagen *et al.* [4]
S. pneumoniae Serogroup 22A/F †	TCTATTAAATAACCCATTGGAATTGAAACG	TCGCAATTGAAGACCACATAAACTG	FAM-TCCGTAATTCGCTTATGGGCACATTCTCCA-NFQ	wcw*V*	Azzari *et al.* [1]
S. pneumoniae Serotype 22F ‡	GAAGATTGTCCACCTTATATCC	TCGGCACAATCAAAATATC	FAM-CGGTTATTT/ZEN/CACAAAAGACACGGTTGG-IABkFQ	wcwA_F	Downs *et al.* [3]
S. pneumoniae Serogroup 23A/B/F †	GGTGGACTTTCCGATGCAA	CACTGTCAACAAAAATGAGGTAATCTC	FAM-AAATGTCGGTATAGATAAAG-NFQ	wchV	Olwagen *et al.* [4]
S. pneumoniae Serotype 23B †	TTGAAGAAATTGCTCCAGAAACAT	CCAAAAGACTAGCCTCAACCACTAA	FAM-TAGAGCTATTTATCTTTCGTGGTTTT-NFQ	wzx	Pholwat *et al.* [2]
S. pneumoniae Serotype 23F †	TGCTATTTGCGATCCTGTTCAT	AGAGCCTCCGTTGTTTCGTAAA	FAM-TTTCTCCGGCATCAAACGTTAAG-NFQ	wzy	Azzari *et al.* [1]
S. pneumoniae Serotype 24A †	CTTGGAGTTGCTAATTATGGGAAG	ATCTCTTACACGTGCACACTC	FAM-CACAGCATATCGTAAAATACCCGCA-NFQ	wzx	Pholwat *et al.* [2]
S. pneumoniae Serogroup 24B/F ‡	TCTGAAAGTAATTAGTAAGATTAACGGAAG	TCCATCTACTTTTAAAATAGCTCCAAC	FAM-CCACAGTCCCAAAATTGTCAGCAACC-BHQ1	wzy	Sakai *et al.* [5]
S. pneumoniae Serogroup 25A/F †	ATACCAACTAGAATCAGCAGGAC	AAATGGAATATCTTTTGATAATTTACTCGC	VIC-CCGCTGGACTTACTGCAATA-NFQ	wcy*E*	Pholwat *et al.* [2]
S. pneumoniae Serogroup 25A/F/38 †	GTCTTACGTAGAACCTCTCTGGATGA	TGGTCCTACAAGCGACATGTG	FAM-TTGCCACAGATTTGGAATATTTTGGTCGG-NFQ	wciI	Olwagen *et al.* [4]
S. pneumoniae Serotype 27 †	AGCGATTTAGCGACTGATATCC	TCTCAAAATCGATCTCGCGTG	FAM-TGTGGAAGGCGTTTGAAGGTGACT-NFQ	wha*K*	Pholwat *et al.* [2]
S. pneumoniae Serogroup 28A/F †	CAACTACAGGTATTTTTGATATCGGAG	GTTTACTACGTTTGTGAAGCGC	FAM-AGAAAATAGTAGGTTGATTGGCGGTGCT-NFQ	wcx*P*	Sakai *et al.* [5]
S. pneumoniae Serotype 29 †	TTCGAGTTGTGCCGTTTTTACA	GGCGTACCCACCTCTAAAATTTT	VIC-TGAATCCTAGTCTTTTCTCTGCG-NFQ	wcr*J*	Pholwat *et al.* [2]
S. pneumoniae Serotype 31 †	GCAGAAGTTTTAAGTCACGGAC	AGCATTACAGATGTCACTAAGGG	FAM-CCCCCACGTAAAACCGCAAGG-NFQ	wzy	Pholwat *et al.* [2]
S. pneumoniae Serogroup 32A/F †	GTACTTCCTGTTCTAGGCTTGG	CCCAGAGGAAAATAGCGTCTC	FAM-TTGTTCAAACCCAACCACTGCTCC-NFQ	wzy	Sakai *et al.* [5]
S. pneumoniae Serogroup 33A/F/37 †	GGAACTGGTTCAGCAACTATACG	GGTTCTAAGACCGTCTGAAATACC	FAM-TAGGACTTTTCTGCCATGCC-NFQ	wzy	Pholwat *et al.* [2]

S. pneumoniae Serogroup 33B/C †	CCTGTTAGTGCACCTGTATTTAAC	GCATTCAAAACTCCTTCATCTCC	FAM-TTCGTTGTTCACGCCATTTA-NFQ	wci*N*	Pholwat *et al.* [2]
S. pneumoniae Serotype 33C †	CAGAGACAGTTTCAGCAAATCTTAG	AGCCTACACCTCTTATAAACGTTG	FAM-CCGTGTCCTATCCACAAACTTGTCTTCC-NFQ	Wzy	Sakai *et al.* [5]
S. pneumoniae Serotype 33D ‡	CGTATAGTCTTGCGACATTTCA	TTCCACATGCGTTACCTCAC	FAM-CACAACTAG/ZEN/TTTTTTATCAAAAAGACCTTGGC-IABkFQ	wci*N*	Pholwat *et al.* [2]
S. pneumoniae Serogroup 34/37/17A †	GGATACTATGTACGAACAGATGGACTTG	CTCACTAACTCGCCCGAATAAAC	FAM-CCGACTATACTCCATTTGA-NFQ	wci*B*	Olwagen *et al.* [4]
S. pneumoniae Serotype 34 †	CGGTGGAGTAGGTCAAGATG	GTCTGTTCTCCCCAATATACTGAG	FAM-ACGGAGCGCCAATGTACTTGAATAGTT-NFQ	Wzy	Pholwat *et al.* [2]
S. pneumoniae Serogroup 35A/C/42 †	TGTTTCAAGCTTCCCCTTTAGA	AAATGAAATCAAAGTATCACGTATCG	FAM-TTCAAAATACCCAGGACACCCGTTCA-NFQ	wcr*K*	Pholwat *et al.* [2]
S. pneumoniae Serotype 35B †	GCATGGAGGTGGAGCATACA	TGTAAAGACTGCACAACTCGATATAAAA	FAM-CAATTTAAACAATATTAGTAAAGCGCAGGTCAAGCAAA-NFQ	wcr*J*	Azzari *et al.* [1]
S. pneumoniae Serogroup 35F/47F †	GTGGTCGTATATACTTGATGAATAAATCG	ACATACAAATTATCAACATACAGATAGGTC	FAM-TTCAACTGGTCGTCCGAATA-NFQ	Wzy	Pholwat *et al.* [2]
S. pneumoniae Serotype 36 †	CTTGTCTATTCAGCCCTTCTGG	CGCGATTATATTGTAAATTGGGAACT	VIC-AGAATGCCCGCTACAATGAG-NFQ	Wzy	Pholwat *et al.* [2]
S. pneumoniae Serotype 39 †	CAAAAAAATGAACTAACTCAAATAGTAACG	ATACTGTAATTTTCTTGTTTATTTGCGG	FAM-AAGTCAGGCGTATTCTTCACAAGGGAAA-NFQ	wcr*G*	Pholwat *et al.* [2]
S. pneumoniae Serotype 41A †	GCAAATAGATGTATCCCAGTTAACAC	GGTAGCTCTTTTGGTTTAATGTCC	FAM-CGACCGAATAGTCTAGCTTCAAAGG-NFQ	wci*B*	Pholwat *et al.* [2]
S. pneumoniae Serotype 41F †	TTTTTGGGAGGAAGTGCTTTT	AACCGCTTTCTCATGATTCATAACT	FAM-CTTCTGTGCTAACAGTGGAGAT-NFQ	Wzx	Pholwat *et al.* [2]
S. pneumoniae Serotype 43 †	AGAGGCTACATCAAATAGTTGGC	GAATCACACCGTAACTTCCAAAG	FAM-TCCAATAGTACTCACCCCTACCGAGC-NFQ	Wzx	Pholwat *et al.* [2]
S. pneumoniae Serotype 45 †	TCTAGCTACTTGACTAAAATATTTGAACTG	GACGAGTCGATTTCGCTGTAT	VIC-CTTTTAGTGACCTCGCTCCC-NFQ	Wzy	Pholwat *et al.* [2]
S. pneumoniae Serotype 46 †	CGAAGTTTTTATATCTCTATTGGTTTG	TATCCCAGGAACTGGACGAA	FAM-TCATTCTTTCTTCAATTCCTTTCTGA-NFQ	Wzy	Sakai *et al.* [8]
S. pneumoniae Serogroup 47A/F †	AGGAATTGGTAGAGAGTTTGTGG	GAAAGTTGAACCATCATCCGTC	FAM-CACTTGATGGAATGCCTGCTGCC-NFQ	whaI	Pholwat *et al.* [2]
S. pneumoniae Serotype 48 †	CAGGTTTTGCTTCATATGGGAG	ATCGGCCAAAAGTTATCATTAGC	FAM-CGCTGCTTATGTGTATTACTCTCCCCTG-NFQ	Wzy	Sakai *et al.* [5]
Acintobacter baumanii †	TTTAGCTCGTCGTATTGGACT	CCTCTTGCTGAGGAGTAATTTT	FAM-TGGCAATGCAGATATCGGTACCCA-NFQ	blaOXA-51-like	Gadsby *et al.*, [9]
Bordetella holmesii †	GGCGACAGCGAGACAGAATC	GCCGCCTTGGCTCACTT	FAM-CGTGCAGATAGGCTTTTAGCTTGAGCGC-NFQ	hIS*1001*	Tatti *et al.*, [10]
B. parapertussis †	TCGAACGCGTGGAATGG	GGCCGTTGGCTTCAAATAGA	FAM-AGACCCAGGGCGCACGCTGTC-NFQ	pIS*1001*	Tatti *et al.*, [10]
B. pertussis/ Holmesii †	CAAGGCCGAACGCTTCAT	GAGTTCTGGTAGGTGTGAGCGTAA	FAM-CAGTCGGCCTTGCGTGAGTGGG-NFQ	IS*481*	Tatti *et al.*, [10]
B. pertussis/ bronchiseptica/ parapertussis †	CGCCAGCTCGTACTTC	GATACGGCCGGCATT	FAM-AATACGTCGACACTTATGGCGA-NFQ	ptx*S1*	Tatti *et al.*, [10]

Escherichia coli †	GTCCAAAGCGGCGATTTG	CAGGCCAGAAGTTCTTTTTC CA	FAM-ACGGCAGAGAAGGTA-NFQ	uid*A*	Lee *et al.,* [11]
Haemophilus influenzae †	CTCAGTTTCGTTTTATTAC CA	CCAGTAACAACAAGGCTA	FAM-CGCATTCTTCTTCGTC CATAACCTTC-NFQ	BexB	This Study
H. influenzae †	CTGAATTRGGYGATTATCT TTATGA	ACAATCAAAYTCAACHGAA AGHGA	FAM-AGGGATGAAA GCYCGRCTTGCAT-NFQ	BexA	Maaroufi *et al,.* [12]
H. influenzae †	CAAAATTGCCAAGATTAA ATGCTT	TGCTCGCCATACTGCACAA	FAM-CCTGCGGTTAAACC-NFQ	IgA1	Olwagen *et al.* [4]
H. influenzae, type b †	TGTTCGCCATAACTTCATC TTAGC	CTTACGCTTCTATCTCGGTG ATTAATAA	FAM-CACAAAACTTCTCAT TCTTCGAGCCTA-NFQ	Bcs3`	Maaroufi *et al.,* [12]
Klebsiella pneumoniae ‡	AGGCCGAATATGACGAAT	GGTGATCTGCTCATGAA	FAM-ACTACCGT CACCCGCCACA-BHQ1	glt*A*	Gadsby *et al.*, [9]
Morexella catarrhalis †	CCGCTTTTACAACCACTG CTT	TGTATCGCCTGCCAAGACAA	FAM-CAGCTGTTAGCCAGCC-NFQ	tonB	Olwagen *et al.* [4]
Neiserria Lactamica †	TTGCCCGAGAACCATTGT ATC	GCGGTTCTTATCACGTTCTAT ATTTG	FAM-TATTGGAGCGGACTAAA-NFQ	lacZ	Olwagen *et al.* [4]
Neiserria meningitidis †	GCACACTTAGGTGATTTA CCTGCAT	CCACCCGTGTGGATCATAAT AGA	FAM-CATGATGGCACAGCA ACAAATCCTGTTT-NFQ	SodC	Dolan Thomas *et al.,* [13]
Pneumocystis jiroveci ‡	CCATCACATCTACGATTAC	GAACGAAATAACCATTGC	FAM-ACTCACATC AACGAGGCGGT BHQ1	Msg-A1	Jensen, (Modified) [14]
Staphylooccus aureus †	GCTCAGCAAATGCATCAC AAA	CACTATATACTGTTGGATCTT CAGAACCA	FAM-AGATAACGGCGTAAATA-NFQ	Tfp	Olwagen *et al.* [4]
Streptococcus pneumoniae ‡	TCTTACGCAATCTAGCAG ATGAAGC	GTTGTTTGGTTGGTTATTCG TGC	FAM-TTTGCCGAAAACG CTTGATACAGGG- BHQ1	LytA	McAvin *et al.*, [15]
S. pneumoniae ‡	AGCAGGTGACTGGTAGGT AAC	CTCCTAATGCTGCTCC	FAM-CAGTTGCTT/ZEN/GCG GTGCACTTG-IABkFQ	Xisco	This Study
S. pneumoniae †	AGCGATAGCTTTCTCCAA GTGG	CTTAGCCAACAAATCGTTTA CCG	FAM-ACCCCAGCAAT TCAAGTGTTCGCG-NFQ	Ply	Greiner *et al.* [16]
S. pneumoniae ‡	CATTGGTGGCTTAGTAAG TGCAA	TACTAACACAAGTTC CTGATAAGGCAAGT	FAM-TGTAAGCGG/ZEN/AAAAG CAGGCCTTACCC-IABkFQ	PiaB	Trzciński *et al.* [17]
S. pyogenes †	GCACTCGCTACTATTTCTT ACCTCAA	GTCACAATGTCTTGGAAACC AGTAAT	FAM-CCGCAACTCATCAAG GATTTCTGTTACCA-NFQ	Spy	CDC 2008; Kodani, [18]
S. algalactidae †	GAACTCTAGTGGCTGGTG CATTG	GGAGTTGTCACTTGATCAGC ATGT	FAM-ATTTTCACCAGCTGTATTAG-NFQ	Cfb	CDC [19]
S. oralis ‡	ACCAGCAGATACGAAAG AAGCAT	AGGTTCGGGCAAGCGATCT TTCT	FAM-AAGGCTGCT/ZEN/GTT GCTGAAGAAGT-IABkFQ	gtf*R*	Alvarez, [20]
Bacterial 16S ribosomal RNA gene ‡	TCCTACGGGAGGCAGCAG T	GGACTACCAGGGTATCTAAT CCTGTT	FAM-CGTATTACC/ZEN/GCG GCTGCTGGCAC-IABkFQ	16S	Nadkarni *et al.*, [21]

Note: † Ordered from Thermofisher; ‡ ordered from Integrated DNA Technologies; NFQ is Nonfluorescent quencher; IABkFQ is an Iowa Black Fluorescent Quencher; BHQ1 is Black Hole Quencher

Nasopharyngeal samples collected from participants at each time point

Table S2. Prevalence of selected nasopharyngeal microbes among participants at baseline, 48 weeks, and 72 weeks

Microbe	Baseline [a]		48 weeks		72 weeks	
	AZM (n = 140)	Placebo (n = 147)	AZM (n = 135)	Placebo (n =133)	AZM (n = 110)	Placebo (n = 95)
Klebsiella pneumoniae	0% (0)	0% (0)	0% (0)	1.5% (2)	0% (0)	0% (0)
Streptococcus pyogenes	2% (3)	1.4% (2)	0% (0)	0.8% (1)	0.9% (1)	0% (0)
Neisseria meningitidis	0% (0)	0.7% (1)	0% (0)	0% (0)	0% (0)	0% (0)
Acinetobacter baumanii	0% (0)	0% (0)	0.7% (1)	0% (0)	0% (0)	0% (0)
RSVA	0.7% (1)	0% (0)	0% (0)	0% (0)	0.9% (1)	1.1% (1)
RSVB	0.7% (1)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Influenza A	0% (0)	0% (0)	0% (0)	0.8% (1)	0.9% (1)	1.1% (1)

Abbreviations: AZM, Azithromycin treatment group; RSVA, respiratory syncytial virus A; RSVB, respiratory syncytial virus B; [a] Fisher's exact test;

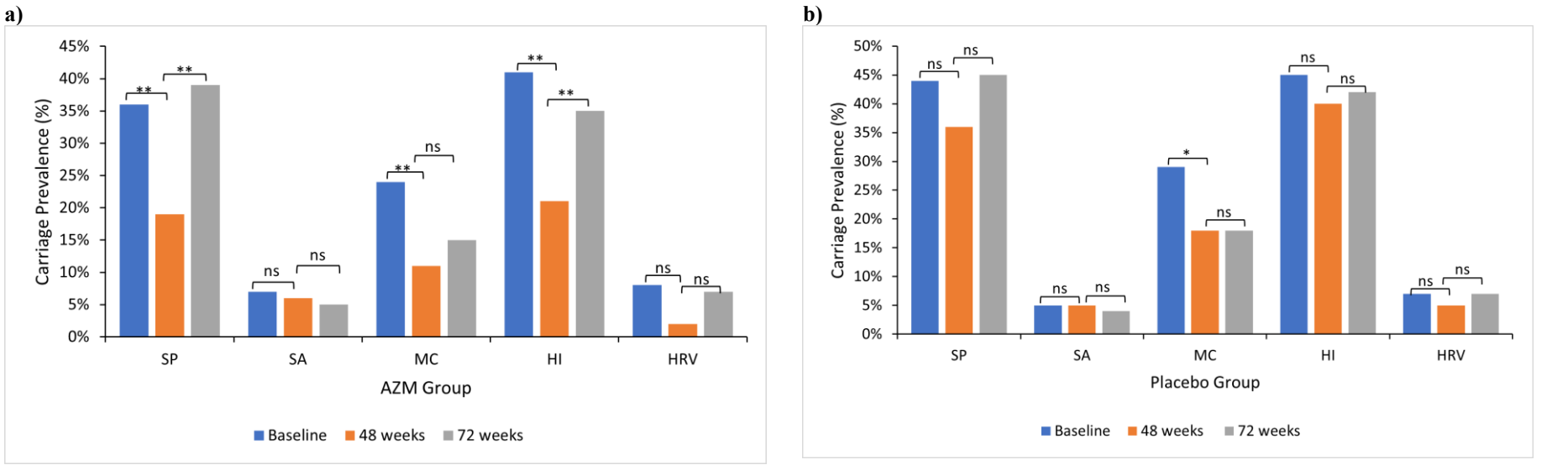

Figure S1. Prevalence of selected microbes at baseline, 48 weeks, and 72 weeks stratified into azithromycin (AZM) and Placebo treatment groups

Abbreviations: AZM, azithromycin; SP, *Streptococcus pneumoniae*; SA, *Staphylococcus aureus*; MC, *Moraxella catarrhalis*; HI, *Haemophilus influenzae*; HRV, Human rhinovirus; Fisher's exact test used to compare 2 visits; logistic mixed effect modelling method using the child as random effect adjusted for age, sex, site, and viral load with visit as the primary outcome; ns- not significant; *, p-value< 0.05, **, p-value <0.01. Results of microbes not detected on all three time points include bacteria (*Klebsiella pneumoniae*, *Neisseria meningitidis*, *Actinobacter baumanii, Streptococcus pyogens)* and viruses (Influenza A, RSVA, and RSVB). *Bordetella pertussis/holmesii*, Influenza B, Human metapneumoviruses, and Human parainfluenza type 1 and 3 were not detected at any of the study visits.

***S. pneumoniae* serotypes distribution and density in participants at baseline, 48 -and 72-weeks**

Overall, 317 serotypes were recovered from both the AZM (n =139) and placebo (n =178) participants at all three time points, with 29 serotypes reported as non-typable (Figure S).

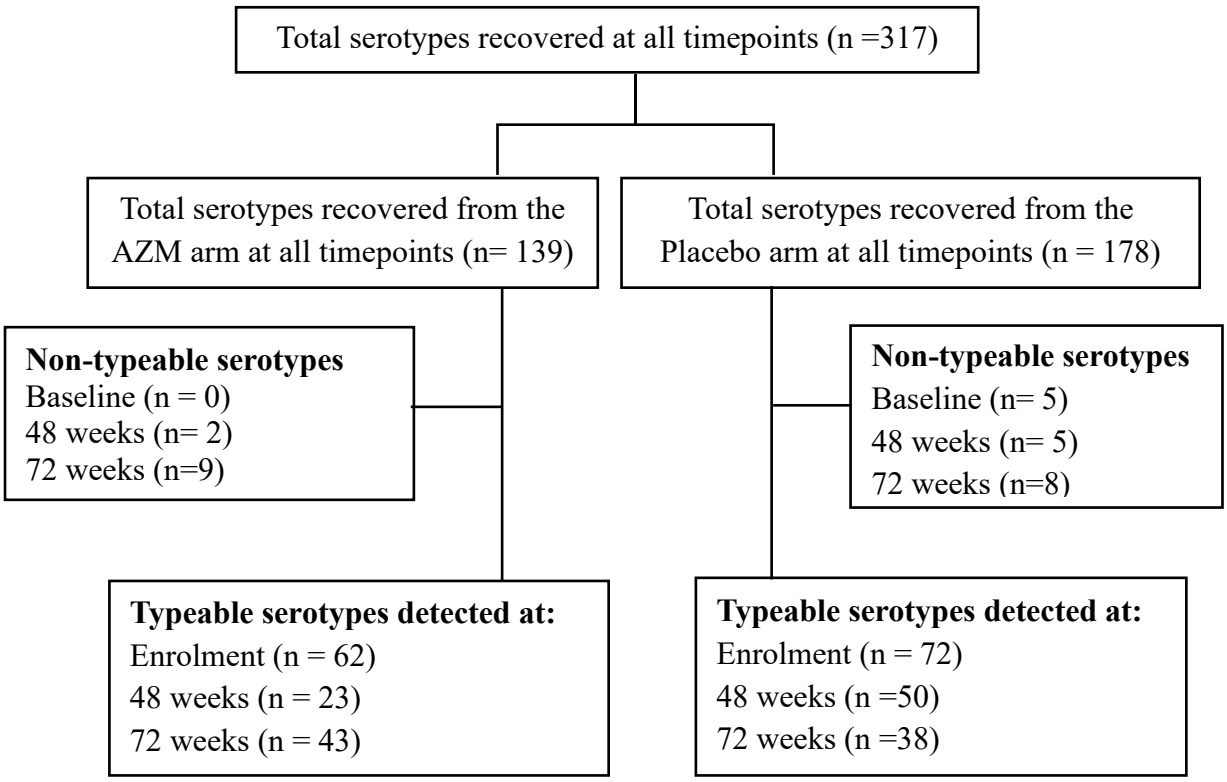

Figure S2. Total serotype numbers detected in nasopharyngeal samples of HIV-infected children and adolescents with CLD at baseline, 48 weeks, and 72 weeks. Non-typeable serotypes are serotypes not included in the nanofluidic qPCR assay (not tested) or were missing during analysis.

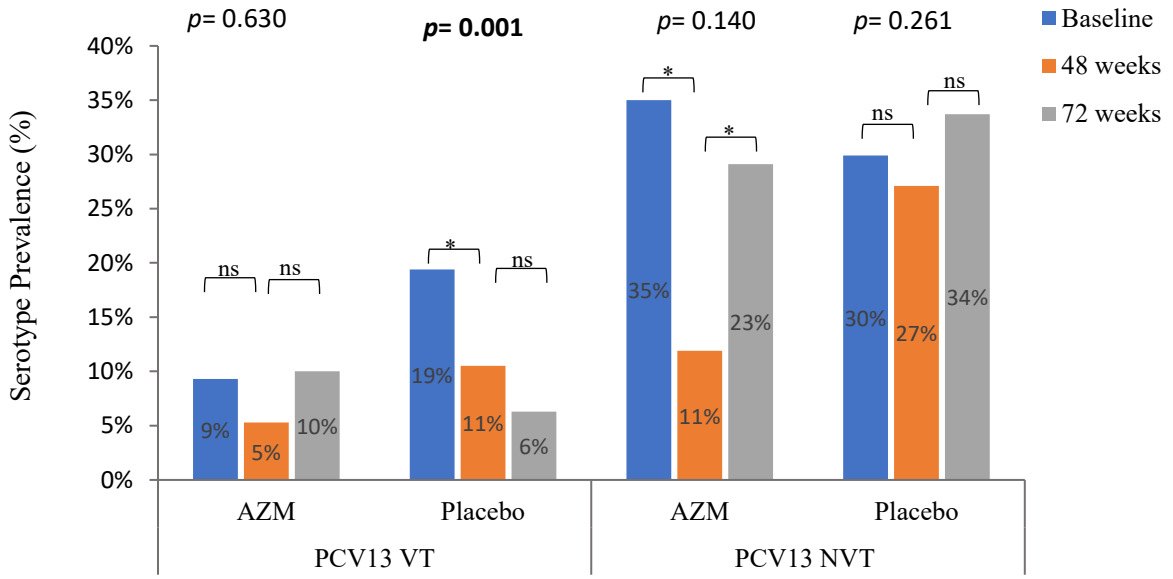

Figure S3. Serotype distribution of *S. pneumoniae* recovered at baseline, 48 weeks and 72 weeks stratified into PCV13 VT serotype and PCV13 NVT serotype groups; P-values were calculated using the logistic mixed effect modelling method using the child as a random effect adjusted for age, sex, site, and viral load, with visit as a primary outcome; ns- not significant; *, p-value< 0.05

Table S3. Baseline Demographic and Clinical Characteristics of Participants Retained versus Lost to Follow-Up or Withdrawn at 48 weeks

Characteristics		**Retained [% (n/N)]**	**Non-retained [% (n/N)]**	***p*-value**
Sociodemographic				
Site	Zimbabwe	77% (156/202)	61% (40/66)	**0.008**
	Malawi	23% (46/202)	29% (43/66)	
Sex	Male	54% (109/202)	39% (26/66)	**0.004**
	Female	46% (93/202)	61% (40/66)	
Age (years)	Median (IQR)	15.4 (12.7 – 17.8)	15.1 (13.2-18.1)	0.667
Anthropometric				
BMI for age Z-score	Median (IQR)	-1.15(-1.86, -0.47)	-0.85 (-1.77, -1.74)	0.220
Current medications				
Taking cotrimoxazole prophylaxis		92% (127/138)	90% (132/66)	0.544
Antiretroviral regimen[m]	NNRT-base-1st line [a]	69% (140/202)	92% (61/66)	**<0.001**
	PI-base 2nd line [b]	31% (62/202)	8% (5/66)	
Clinical parameters				
CD4 count categories (Cells/mm)	< 200	9% (18/202)	18% (12/66)	0.142
	200-500	25% (51/202)	29% (19/66)	
	>500	66% (133/202)	53% (35/66)	
Viral load suppression	VL < 1000 copies/mL	63% (124/196)	59% (38/66)	0.577
Previously treated for tuberculosis		66% (133/202)	18% (12/66)	**0.014**

P-values were calculated using Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables

Table S4. *S. pneumoniae* serotypes recovered in participants by study arm and timepoint

Group	**Baseline**		**48 weeks**		**72 weeks**	
	PCV13	NVT13	PCV13	NVT13	PCV13	NVT13
Single serotype						
AZM	13	29	7	13	9	21
Placebo	25	21	11	16	6	20
>2 serotypes						
AZM	0	8	0	3	1	6
Placebo	2	9	2	9	1	6
≥2 serotypes						
AZM	0	2	0	1	0	1
Placebo	0	0	0	2	0	1

Table S5. Pneumococcal serotypes recovered at all three time points

Serotype	**Baseline** [a]		**48 weeks** [b]		**72 weeks** [b]	
	AZM (n= 140)	Placebo (n=147)	AZM (n = 135)	Placebo (n = 133)	AZM (n = 110)	Placebo (n = 95)
3	0.7% (1)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
4	1.4% (2)	3.4% (5)	0% (0)	1.5% (2)	0% (0)	1.1% (1)
5	0% (0)	0.7% (1)	0% (0)	0% (0)	0.9% (1)	0 (0)
6A	0% (0)	0.7% (1)	0% (0)	0.8% (1)	0% (0)	1.1% (1)
6B	0% (0)	0.7% (1)	0% (0)	0.8% (1)	0% (0)	0% (0)
7AF	0% (0)	1.4% (2)	0% (0)	0% (0)	0% (0)	0% (0)
9AV	0.7% (1)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
14	0.7% (1)	2% (3)	0.7% (1)	0.8% (1)	1.8% (2)	0% (0)
18C	0.7% (1)	2% (3)	0% (0)	0.8% (1)	0% (0)	0% (0)
19A	0.7% (1)	4.1% (6)	0% (0)	0.8% (1)	0% (0)	1.1% (1)
19F	1.4% (2)	3.4% (5)	2.2% (3)	3% (4)	4.5% (5)	1.1% (1)
23F	2.9% (4)	0.7% (1)	2.2% (3)	2.3% (3)	2.7% (3)	2.1% (2)
6C	0% (0)	0.7% (1)	0% (0)	0.8% (1)	0% (0)	2.1% (2)
7BC/40	1.4% (2)	2% (3)	0% (0)	2.3% (3)	0% (0)	1.1% (1)
8	0% (0)	0% (0)	0% (0)	0% (0)	0.9% (1)	0% (0)
9LN	1.4% (2)	0.7% (1)	0.7% (1)	0% (0)	0.9% (1)	1.1% (1)
10A	0% (0)	0% (0)	0% (0)	0% (0)	1.8% (2)	0% (0)
11AD	1.4% (2)	2% (3)	0.7% (1)	0.8% (1)	0% (0)	3.2% (3)
11E	0% (0)	0% (0)	0.7% (1)	2.3% (3)	0.9% (1)	1.1% (1)
11 like	1.4% (2)	0 % (0)	0% (0)	0% (0)	0% (0)	0% (0)
12 grp/44/46	2.1% (3)	0% (0)	0% (0)	0.8% (1)	1.8% (2)	1.1% (1)
13	2.1% (3)	2.7% (4)	0% (0)	1.5% (2)	2.7% (3)	0% (0)
15AF	2.1% (3)	1.4% (2)	0.7% (1)	3% (4)	1.8% (2)	2.1% (2)
15 BC	0.7% (1)	1.4% (2)	0% (0)	0% (0)	0% (0)	1.1% (1)
15 like	0.7% (1)	0.7% (1)	0.7% (1)	2.3% (3)	2.7% (3)	1.1% (1)
16A	1.4% (2)	1.4% (2)	0% (0)	0.8% (1)	0% (0)	2.1% (2)
16F	2.9% (4)	0.7% (1)	0% (0)	1.5% (2)	1.8% (2)	3.2% (3)
17F	0.7% (1)	1.4% (2)	0% (0)	0.8% (1)	0.9% (1)	0% (0)
18A	0.7% (1)	0.7% (1)	1.5% (2)	0.8% (1)	0% (0)	0% (0)
18B	0% (0)	0.7% (1)	0% (0)	0.8% (1)	0% (0)	1.1% (1)
19B	0.7% (1)	1.4% (2)	0.7% (1)	0.8% (1)	0.9% (1)	1.1% (1)

19C	0.7% (1)	1.4% (2)	0% (0)	0% (0)	0% (0)	0% (0)
19 atypical	0% (0)	0.7% (1)	0% (0)	0% (0)	0% (0)	0% (0)
20	0% (0)	0.7% (1)	1.5% (2)	0% (0)	0.9% (1)	0% (0)
21	2.1% (3)	3.4% (5)	0% (0)	1.5% (2)	0% (0)	1.1% (1)
22A	0% (0)	0% (0)	0.7% (1)	0% (0)	0.9% (1)	1.1% (1)
23A	1.4% (2)	0.7% (1)	0.7% (1)	1.5% (2)	0.9% (1)	0% (0)
23B	1.4% (2)	0% (0)	0% (0)	1.5% (2)	1.8% (2)	1.1% (1)
24A	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	1.1% (1)
27	0% (0)	0.7% (1)	0% (0)	0% (0)	0% (0)	0% (0)
28AF	2.1% (3)	0% (0)	1.5% (2)	0% (0)	0.9% (1)	1.1% (1)
29	0.7% (1)	0% (0)	0% (0)	0.8% (1)	0.9% (1)	0% (0)
31	0% (0)	0.7% (1)	0% (0)	0% (0)	0% (0)	0% (0)
33C	1.4% (2)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
34	2.1% (3)	1.4% (2)	0% (0)	1.5% (2)	0% (0)	1.1% (1)
35AC/42	0% (0)	0.7% (1)	0.7% (1)	0% (0)	1.8% (2)	0% (0)
35B	0% (0)	0% (0)	0.7% (1)	0% (0)	0.9% (1)	0% (0)
35F	0% (0)	0% (0)	0.7% (1)	0% (0)	0.9% (1)	1.1% (1)
38	1.4% (2)	0% (0)	0.7% (1)	0% (0)	0.9% (1)	2.1% (2)
41A	0% (0)	0% (0)	0% (0)	0% (0)	0.9% (1)	0% (0)
43	1.4% (2)	0.7% (1)	0% (0)	1.5% (2)	0% (0)	1.1% (1)
45	0% (0)	0.7% (1)	0% (0)	0.8% (1)	0% (0)	0% (0)
47A	0% (0)	0.7% (1)	0% (0)	1.5% (2)	0% (0)	1.1% (1)
48	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	1.1% (1)

Abbreviations: AZM, AZM treatment group; [b] Fisher's exact test; [c] logistic mixed effect modelling method using the child as random effect adjusted for age, sex, site, visit and viral load. No individual serotypes were statistically significant.

References

1. Azzari C, Moriondo M, Indolfi G, Cortimiglia M, Canessa C, Becciolini L, Lippi F, de Martino M, Resti M: Realtime PCR is more sensitive than multiplex PCR for diagnosis and serotyping in children with culture negative pneumococcal invasive disease. PLoS One 2010, 5(2):e9282.
2. Pholwat S, Sakai F, Turner P, Vidal JE, Houpt ER: Development of a TaqMan Array Card for Pneumococcal Serotyping on Isolates and Nasopharyngeal Samples. J Clin Microbiol 2016, 54(7):1842-1850.
3. Downs SL, Madhi SA, Van der Merwe L, Nunes MC, Olwagen CP: High-throughput nanofluidic real-time PCR to discriminate Pneumococcal Conjugate Vaccine (PCV)-associated serogroups 6, 18, and 22 to serotypes using modified oligonucleotides. Scientific Reports 2021, 11(1):23728.
4. Olwagen CP, Adrian PV, Madhi SA: Comparison of traditional culture and molecular qPCR for detection of simultaneous carriage of multiple pneumococcal serotypes in African children. Sci Rep 2017, 7(1):4628.
5. Sakai F, Sonaty G, Watson D, Klugman KP, Vidal JE: Development and characterization of a synthetic DNA, NUversa, to be used as a standard in quantitative polymerase chain reactions for molecular pneumococcal serotyping. FEMS Microbiol Lett 2017, 364(17).
6. Messaoudi M, Milenkov M, Albrich WC, van der Linden MPG, Bénet T, Chou M, Sylla M, Barreto Costa P, Richard N, Klugman KP, Endtz HP, Paranhos-Baccalà G, Telles J-N: The Relevance of a Novel Quantitative Assay to Detect up to 40 Major Streptococcus pneumoniae Serotypes Directly in Clinical Nasopharyngeal and Blood Specimens. PLoS One 2016, 11(3):e0151428.
7. Downs SL, Madhi SA, van der Merwe L, Nunes MC, Olwagen CP: Optimization of a high-throughput nanofluidic real-time PCR to detect and quantify of 15 bacterial species and 92 Streptococcus pneumoniae serotypes. Scientific Reports 2023, 13(1):4588.
8. Sakai F, Chochua S, Satzke C, Dunne EM, Mulholland K, Klugman KP, Vidal JE: Single-plex quantitative assays for the detection and quantification of most pneumococcal serotypes. PLoS One 2015, 10(3):e0121064.
9. Gadsby NJ, McHugh MP, Russell CD, Mark H, Conway Morris A, Laurenson IF, Hill AT, Templeton KE: Development of two real-time multiplex PCR assays for the detection and quantification of eight key bacterial pathogens in lower respiratory tract infections. Clinical Microbiology and Infection 2015, 21(8):788.e781-788.e713.
10. Tatti KM, Sparks KN, Boney KO, Tondella ML: Novel Multitarget Real-Time PCR Assay for Rapid Detection of Bordetella Species in Clinical Specimens. Journal of Clinical Microbiology 2011, 49(12):4059.
11. Lee D-Y, Shannon K, Beaudette LA: Detection of bacterial pathogens in municipal wastewater using an oligonucleotide microarray and real-time quantitative PCR. Journal of Microbiological Methods 2006, 65(3):453-467.
12. Maaroufi Y, De Bruyne J-M, Heymans C, Crokaert F: Real-Time PCR for Determining Capsular Serotypes of Haemophilus influenzae. Journal of Clinical Microbiology 2007, 45(7):2305.

13. Dolan Thomas J, Hatcher CP, Satterfield DA, Theodore MJ, Bach MC, Linscott KB, Zhao X, Wang X, Mair R, Schmink S, Arnold KE, Stephens DS, Harrison LH, Hollick RA, Andrade AL, Lamaro-Cardoso J, de Lemos APS, Gritzfeld J, Gordon S, Soysal A *et al*: sodC-Based Real-Time PCR for Detection of Neisseria meningitidis. PLoS One 2011, 6(5):e19361.
14. Jensen L, Jensen AV, Praygod G, Kidola J, Faurholt-Jepsen D, Changalucha J, Range N, Friis H, Helweg-Larsen J, Jensen JS, Andersen AB: Infrequent detection of Pneumocystis jirovecii by PCR in oral wash specimens from TB patients with or without HIV and healthy contacts in Tanzania. BMC Infectious Diseases 2010, 10(1):140.
15. McAvin JC, Reilly PA, Roudabush RM, Barnes WJ, Salmen A, Jackson GW, Beninga KK, Astorga A, McCleskey FK, Huff WB, Niemeyer D, Lohman KL: Sensitive and specific method for rapid identification of Streptococcus pneumoniae using real-time fluorescence PCR. J Clin Microbiol 2001, 39(10):3446-3451.
16. Greiner O, Day PJR, Bosshard PP, Imeri F, Altwegg M, Nadal D: Quantitative Detection of Streptococcus pneumoniae in Nasopharyngeal Secretions by Real-Time PCR. Journal of Clinical Microbiology 2001, 39(9):3129-3134.
17. Trzciński K, Bogaert D, Wyllie A, Chu MLJN, van der Ende A, Bruin JP, van den Dobbelsteen G, Veenhoven RH, Sanders EAM: Superiority of Trans-Oral over Trans-Nasal Sampling in Detecting Streptococcus pneumoniae Colonization in Adults. PLoS One 2013, 8(3):e60520.
18. Kodani M, Yang G, Conklin LM, Travis TC, Whitney CG, Anderson LJ, Schrag SJ, Taylor TH, Beall BW, Breiman RF, Feikin DR, Njenga MK, Mayer LW, Oberste MS, Tondella MLC, Winchell JM, Lindstrom SL, Erdman DD, Fields BS: Application of TaqMan Low-Density Arrays for Simultaneous Detection of Multiple Respiratory Pathogens. Journal of Clinical Microbiology 2011, 49(6):2175.
19. CDC: Centers for Disease Control and Prevention, Streptococcus Laboratory Protocols. Accessed 30-Jan-2016.
20. Àlvarez G, González M, Isabal S, Blanc V, León R: Method to quantify live and dead cells in multi-species oral biofilm by real-time PCR with propidium monoazide. AMB Express 2013, 3(1):1.
21. Nadkarni MA, Martin FE, Jacques NA, Hunter N: Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology 2002, 148(1):257-266.