Oligonucleotides used in the multiplex real-time PCR detection of antibiotic resistance and pili genes from clinical specimens or isolates confirmed as <u>positive</u> for *Streptococcus pneumoniae* by culture or *lyt*A PCR.

<u>Reference</u>: Velusamy et al. (2020) Expanded sequential quadriplex real-time polymerase chain reaction (PCR) for identifying pneumococcal serotypes, penicillin susceptibility, and resistance markers. Diagnostic Microbiology and Infectious Disease 97(2): 115037. doi: 10.1016/j.diagmicrobio.2020.115037. Epub 2020 Mar 12.

Antibiotic Resistance and Pilus Genes									
tetM-F	GCTTATACTATAGCCCTGTTAGTACC	300				*tetM* (NG_048253)	1611-1714	104	Velusamy et al. 2020
tetM-P	TCGGATGCTTGCTCCTATTGTATTGGA	100	CY5		BHQ3				
tetM-R	TGGCTCTAACAATTCTGTTCCA	300							
ermB-F	GCAATTGCTTAAGCTGCCA	300				*ermB* (AP018043)	622813-622717	97	Velusamy et al. 2020
ermB-P	TGGTTTAGGATGAAAGCATTCCGC	300	FAM		BHQ1				
ermB-R	ATCTGGAACATCTGTGGTATGG	300							
pbp2b-F*	GGCTGTTTGGACCATATAGGTATT	200				*pbp2b* (AE007317)	1494983-1494903	81	Velusamy et al. 2020
pbp2b-P	CAGAGCTTGGACCGCTGTGATAGG	200	ROX		BHQ2				
pbp2b-R	ACTCAGGCTTACGGTTCATTC	200							
mef-F	GAGCTACCTGTCTGGATGATTATG	400				*mef* (CP000921)	1803434-1803342	93	Velusamy et al. 2020
mef-P	TGTTTATCCGTAGCATTGGAACAGCT	200	HEX		BHQ1				
mef-R	AAGTGGTGTAACCGCATTGA	400							
Cat-F	GTGACAAGGGTGATAAACTCAAATAC	200				*cat* (AP018043)	652571-652657	87	Velusamy et al. 2020
Cat-P	ACTGGTTACAATAGCGACGGAGAGT	200	ROX		BHQ2				
Cat-R	TGGCTCTAACTTATCCCAATAACC	200							
Pilus1-F	GATATTTCAGAAGGCAGTTGCAG	200				Pilus1 (CP000921)	463594-463979	83	Velusamy et al. 2020
Pilus1-P	ACTGTGCTGTATATCTCAGTTGACAGCT	200	FAM		BHQ1				
Pilus1-R	GCGTTTCTGCTAAAGCAACTATC	200							
Pilus2-F	TGCAAGCATTGCAGCTA	400				Pilus2 (CP000921)	1003538-1003616	79	Velusamy et al. 2020
Pilus2-P	TCCGTATTCAGAGGTGTCCCGACT	100	HEX		BHQ1				
Pilus2-R	GGTTATTGCTGAATTATCATCCG	400							

*-*pbp2b* (penicillin) gene targeted for susceptibility

Interpretation criteria for Antibiotic Resistance and pilus genes

Antibiotic Resistance Gene*	Target	Interpretation
pbp2B	SPN Penicillin susceptibility	Should be interpreted as Penicillin sensitivity. If PCR is negative then Penicillin non-susceptible
ermB	SPN Erythromycin and Clindamycin resistance	Resistant to Erythromycin and Clindamycin
mef	SPN Erythromycin resistance	Resistant to Erythromycin
tetM	SPN Tetracycline resistance	Resistant to Tetracycline
cat	SPN Chloramphenicol resistance	Resistant to Chloramphenicol