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

**Reference**: 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.

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| **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**

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| **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 |