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| **Table S1. Primers used for PCR serotyping of pneumococci recovered from children in Nepal.** |
|  |  |  |  |  |  |
| **Serotype** | **Primer name** | **Sequence (5’ – 3’)** | **Amplicon (bp)** | **Reference** | **Comments** |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
| 1 | 1-f | CTC TAT AGA ATG GAG TAT ATA AAC TAT GGT TA | 280 | 1, 2 | Blast hits to 1 |
|  | 1-r | CCA AAG AAA ATA CTA ACA TTA TCA CAA TAT TGG C |  |  |  |
|  |  |  |  |  |  |
| 2 | 2F | GTC ATT GTT ACG ATT AGT TTC GAT AGT TGA GG | 381 | 3 | Blast hits to 2 |
|  | 2R | AAT TCA ATT CCT AAG TCC TCT TCC ATA AAC TC |  |  |  |
|  |  |  |  |  |  |
| 3 | 3-f | ATG GTG TGA TTT CTC CTA GAT TGG AAA GTA G | 371 | 1, 2 | Blast hits to 3 |
|  | 3-r | CTT CTC CAA TTG CTT ACC AAG TGC AAT AAC G |  |  |  |
|  |  |  |  |  |  |
| 4 | 4-f | CTG TTA CTT GTT CTG GAC TCT CGA TAA TTG G | 430 | 1, 2 | Blast hits to 4 |
|  | 4-r | GCC CAC TCC TGT TAA AAT CCT ACC CGC ATT G |  |  |  |
|  |  |  |  |  |  |
| 5 | 5-f | ATA CCT ACA CAA CTT CTG ATT ATG CCT TTG TG | 362 | 1, 2 | Blast hits to 5 |
|  | 5-r | GCT CGA TAA ACA TAA TCA ATA TTT GAA AAA GTA TG |  |  |  |
|  |  |  |  |  |  |
| 6 | 6A/6B/6C/6D-f | AAT TTG TAT TTT ATT CAT GCC TAT ATC TGG | 250 | 1, 2 | Blast hits to group 6 |
|  | 6A/6B/6C/6D-r | TTA GCG GAG ATA ATT TAA AAT GAT GAC TA |  |  |  |
|  |  |  |  |  |  |
| 7B/C, 40 | 7C/7B/40-f | CTA TCT CAG TCA TCT ATT GTT AAA GTT TAC GAC GGG A | 260 | 1, 2 | Blast hits 7B/C and 40  |
|  | 7C/7B/40-r | GAA CAT AGA TGT TGA GAC ATC TTT TGT AAT TTC |  |  |  |
|  |  |  |  |  |  |
| 7F/A | 7F-f | CCT ACG GGA GGA TAT AAA ATT ATT TTT GAG | 826 | 1, 2 | Blast hits to 7F and 7A |
|  | 7F-r | CAA ATA CAC CAC TAT AGG CTG TTG AGA CTA AC |  |  |  |
|  |  |  |  |  |  |
| 8 | 8-f | GAT GCC ATG AAT CAA GCA GTG GCT ATA AAT C | 294 | 1, 2 | Blast hits to 8 |
|  | 8-r | ATC CTC GTG TAT AAT TTC AGG TAT GCC ACC |  |  |  |
|  |  |  |  |  |  |
| 9N/L | 9N/9L-f | GAA CTG AAT AAG TCA GAT TTA ATC AGC | 516 | 1, 4 | Blast hits to 9N and 9L |
|  | 9N/9L-r | ACC AAG ATC TGA CGG GCT AAT CAA T |  |  |  |
|  |  |  |  |  |  |
| 9V/A | 9V-f | CTT CGT TAG TTA AAA TTC TAA ATT TTT CTA AG | 753 | 1, 2 | Blast hits to 9V and 9A |
|  | 9V-r | GTC CCA ATA CCA GTC CTT GCA ACA CAA G |  |  |  |
|  |  |  |  |  |  |
| 9V/A | 9V/9A-f | GGG TTC AAA G TC AGA CAG TG A ATC TTA A | 816 | 1, 5 | Blast hits to 9V and 9A |
|  | 9V/9A-r | CCA TGA ATG A AA TCA ACA TT G TCA GTA GC |  |  |  |
|  |  |  |  |  |  |
| 10A | 10A-f | GGT GTA GAT TTA CCA TTA GTG TCG GCA GAC | 628 | 1, 2 | Blast hits to 10A |
|  | 10A-r | GAA TTT CTT CTT TAA GAT TCG GAT ATT TCT C |  |  |  |
|  |  |  |  |  |  |
| 10F/C, 33C | 10F/10C/33C-f | GGA GTT TAT CGG TAG TGC TCA TTT TAG CA | 248 | 1, 5 | Blast hits to 10F/C and 33C (or new33E) |
|  | 10F/10C/33C-r | CTA ACA AAT TCG CAA CAC GAG GCA ACA |  |  |  |
|  |  |  |  |  |  |
| 11 | 11A/11D-f | GGA CAT GTT CAG GTG ATT TCC CAA TAT AGT G | 463 | 1, 2 | Blast hits to new 11E, and 11D, 11A, 11F |
|  | 11A/11D-r | GAT TAT GAG TGT AAT TTA TTC CAA CTT CTC CC |  |  |  |
|  |  |  |  |  |  |
| 12A/B/F, 44, 46  | 12A/F-F | ACT CTT CCA AAT TCT TAT GCT TTT ATT GAT TC | 656 | 3 | Blast hits to 12A/B/F, 44, 46 |
|  | 12A/F-R | ATG AAT GAG AAA AGG AAC TTA AAA TTC ATA GC |  |  |  |
|  |  |  |  |  |  |
| 13 | 13-f | TAC TAA GGT AAT CTC TGG AAA TCG AAA GG | 655 | 1, 5 | Blast hits to 13 and new serotype (subtype |
|  | 13-r | CTC ATG CAT TTT ATT AAC CG C TTT TTG TTC |  |  | of 20 with 1 mismatch in F); R to 13 |
|  |  |  |  |  |  |
| 14 | 14-f | CTT GGC GCA GGT GTC AGA ATT CCC TCT AC | 208 | 1, 2 | Blast hits to 14; can cross-react with 15B/C |
|  | 14-r | GCC AAA ATA CTG ACA AAG CTA GAA TAT AGC C |  |  |  |
|  |  |  |  |  |  |
| 15A/F | 15A/15F-f | ATT AGT ACA GCT GCT GGA ATA TCT CTT C | 434 | 1, 2 | Blast hits to 15A, 15F |
|  | 15A/15F-r | GAT CTA GTG AAC GTA CTA TTC CAA AC |  |  |  |
|  |  |  |  |  |  |
| 15B/C | 15B/15C-F | AGG AAT CAG ATA TTA TCA TTA CTC ATG GTG  | 496 | 3 | Can cross react with 14 |
|  | 15B/15C-R | TCA TGA CCC ATA GAA CTA TAT AAA AAG ACG  |  |  |  |
|  |  |  |  |  |  |
| 16F | 16F-f | GAA TTT TTC AGG CGT GGG TGT TAA AAG | 717 | 1, 5 | Blast hits to 16F |
|  | 16F-r | CAG CAT ATA GCA CCG CTA AGC AAA TA |  |  |  |
|  |  |  |  |  |  |
| 17F | 17F-f | TTC GTG ATG ATA ATT CCA ATG ATC AAA CAA GAG | 693 | 1, 2 | Blast hits to 17F |
|  | 17F-r | GAT GTA ACA AAT TTG TAG CGA CTA AGG TCT GC |  |  |  |
|  |  |  |  |  |  |
| 18 | 18C/18F/18B/18A-f | CTT AAT AGC TCT CAT TAT TCT TTT TTT AAG CC | 573 | 1, 2 | Blast hits to 18 |
|  | 18C/18F/18B/18A-r | TTA TCT GTA AAC CAT ATC AGC ATC TGA AAC |  |  |  |
|  |  |  |  |  |  |
| 19A | 19A-f | GTT AGT CCT GTT TTA GAT TTA TTT GGT GAT GT | 478 | 1, 2 | Blast hits to 19A |
|  | 19A-r | GAG CAG TCA ATA AGA TGA GAC GAT AGT TAG |  |  |  |
|  |  |  |  |  |  |
| 19F | 19F-f | GTT AAG ATT GCT GAT CGA TTA ATT GAT ATC C | 304 | 1, 2 | Blast hits to 19F |
|  | 19F-r | GTA ATA TGT CTT TAG GGC GTT TAT GGC GAT AG |  |  |  |
|  |  |  |  |  |  |
| 20 | 20-f | GAG CAA GAG TTT TTC ACC TGA CAG CGA GAA G | 514 | 1, 2 | Blast hits to 20, possible new serotype |
|  | 20-r | CTA AAT TCC TGT AAT TTA GCT AAA ACT CTT ATC |  |  | within serogroup 20 |
|  |  |  |  |  |  |
| 21 | 21-f | CTA TGG TTA TTT CAA CTC AAT CGT CAC C | 192 | 1, 5 | Blast hits to 21 |
|  | 21-r | GGC AAA CTC AGA CAT AGT ATA GCA TAG |  |  |  |
|  |  |  |  |  |  |
| 22F/A | 22F/22A-f | GAG TAT AGC CAG ATT ATG GCA GTT TTA TTG TC | 643 | 1, 2 | Blast hits to 22F/A |
|  | 22F/22A-r | CTC CAG CAC TTG CGC TGG AAA CAA CAG ACA AC |  |  |  |
|  |  |  |  |  |  |
| 23A | 23A-f | TAT TCT AGC AAG TGA CGA AGA TGC G | 722 | 1, 5 | Blast hits to 23A |
|  | 23A-r | CCA ACA TGC TTA AAA ACG CTG CTT TAC |  |  |  |
|  |  |  |  |  |  |
| 23B | 23B-F | TTG TTA GTG GTA TTA AAT TGG GGA CTA CTA GG | 216 | 3 | Blast hits to 23B |
|  | 23B-R | ATA CCT ATC TGA AGT GTT ATT AAC CCA CCA AC |  |  |  |
|  |  |  |  |  |  |
| 23F | 23F-f | GTA ACA GTT GCT GTA GAG GGA ATT GGC TTT TC | 384 | 1, 2 | Blast hits to 23F |
|  | 23F-r | CAC AAC ACC TAA CAC TCG ATG GCT ATA TGA TTC |  |  |  |
|  |  |  |  |  |  |
| 24A/F | 24A/F-F | TCT CAA CCA AGA TAC AGA TTT TGA TTT TAC TC | 686 | 3 | Blast hits to 24A/F |
|  | 24A/F-R | TAT AAA CCT TTA GTA AAC ACT CTG CTT GAT CG |  |  |  |
|  |  |  |  |  |  |
| 29 | 29F | ATT ATC TCG GAT CAA ACA ATT CTT TTG TAA AC | 259 | 3 | Blast hits to 29 |
|  | 29R | AAC GCT AAC ATT AAA ACT AGA ACG AGT AAA CC |  |  |  |
|  |  |  |  |  |  |
| 31 | 31-f | GGA AGT TTT CAA GGA TAT GAT AGT GGT GGT GC | 701 | 1, 2 | Blast hits to 31 |
|  | 31-r | CCG AAT AAT ATA TTC AAT ATA TTC CTA CTC |  |  |  |
|  |  |  |  |  |  |
| 33F/A, 37 | 33F/33A/37-f | GAA GGC AAT CAA TGT GAT TGT GTC GCG | 338 | 1, 2 | Blast hits to 33F/A, 37 |
|  | 33F/33A/37-r | CTT CAA AAT GAA GAT TAT AGT ACC CTT CTA C |  |  |  |
|  |  |  |  |  |  |
| 34 | 34-f | GCT TTT GTA AGA GGA GAT TAT TTT CAC CCA AC | 408 | 1, 2 | Blast hits to 34 |
|  | 34-r | CAA TCC GAC TAA GTC TTC AGT AAA AAA CTT TAC |  |  |  |
|  |  |  |  |  |  |
| 35A/C, 42 | 35A/35C/42-f | ATT ACG ACT CCT TAT GTG ACG CGC ATA | 280 | 1, 5 | Blast hits to 35A/C, 42 |
|  | 35A/35C/42-r | CCA ATC CCA AGA TAT ATG CAA CTA GGT T |  |  |  |
|  |  |  |  |  |  |
| 35B | 35B-f | GAT AAG TCT GTT GTG GAG ACT TAA AAA GAA TG | 677 | 1, 2 | Blast hits to 35B |
|  | 35B-r | CTT TCC AGA TAA TTA CAG GTA TTC CTG AAG CAA G |  |  |  |
|  |  |  |  |  |  |
| 35F, 47F | 35F/47F-f | GAA CAT AGT CGC TAT TGT ATT TTA TTT AAA GCA A | 517 | 1, 2 | Blast hits to 35F, 47F |
|  | 35F/47F-r | GAC TAG GAG CAT TAT TCC TAG AGC GAG TAA ACC |  |  |  |
|  |  |  |  |  |  |
| 38, 25F/A | 38/25F/25A-f | CGT TCT TTT ATC TCA CTG TAT AGT ATC TTT ATG | 574 | 1, 2 | Blast hits to 38, 25F/A |
|  | 38/25F/25A-r | ATG TTT GAA TTA AAG CTA ACG TAA CAA TCC |  |  |  |
|  |  |  |  |  |  |
| 39 | 39-f | TCA TTG TAT TAA CCC TAT GCT TTA TTG GTG | 98 | 1, 5 | Blast hits to 39 |
|  | 39-r | GAG TAT CTC CAT TGT ATT GAA ATC TAC CAA |  |  |  |
|  |  |  |  |  |  |
| 45 | 45-f | GTT TAA TGG CTG ATG AAG TTA TTA TTG TTG | 238 | 3 | Blast hits to 45 |
|  | 45-r | TTT ACC ATC AGT GAA ATT TTA TCT TTG TTC |  |  |  |
|  |  |  |  |  |  |
| *cpsA* | *cpsA*-f | GCA GTA CAG CAG TTT GTT GGA CTG ACC | 160 | 1, 2 | Used in every PCR reaction |
|  | *cpsA*-r | GAA TAT TTT CAT TAT CAG TCC CAG TC |  |  |  |

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