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Streptococcus pneumoniae detection based in the *cpsA* and *cpsC* genes will miss serotypes 25A, 25F and 38

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Background

Traditionally, *Streptococcus pneumoniae* serotyping has been substantiated in serological methods such as Quellung reaction which continues being the gold standard. This method requires expertise so molecular techniques such amplification of the *cpsA*, *lytA* and/or *ply* genes has become in a routinely method for pneumococcal detection. Over the past five years, the pneumococcal conjugate vaccine usage has led to a serotype replacement in both early and late vaccination period (PCV7 and PCV13). One of this emerging serotype is the 38, indeed, it has been observed an increase of this serotype causing pneumococcal invasive disease in children. However, serotypes 38 and 25A and 25F could be misidentified because their mutation in *cpsA* gene. Aim: Evaluate pneumococcus detection based only in amplification of *cpsA*, *lytA*, *ply* genes .

Material and Methods

Prospective study including the first optochin susceptibility 124 pneumococci (9 invasive and 115 non-invasive) isolated from clinical samples at Donostia University Hospital, San Sebastián, Spain, during the first months of 2015.

Presumed pneumococcal was identified by the optochin test and then confirmed by a multiplex PCR of the *cpsA*, *lytA*, and *ply* genes.

Serotyping was performed by: a) a multiplex PCR combining the fragment analysis by fluorescence labelled-primers b) a recently developed PCR/hybridization assay which identified 42 different serotypes besides the *cpsA*, *lytA*, and *ply* genes by specific hybridization c) Quellung reaction.

Figure 1. Geographic location and Donostia University Hospital and Microbiology Service center of out study

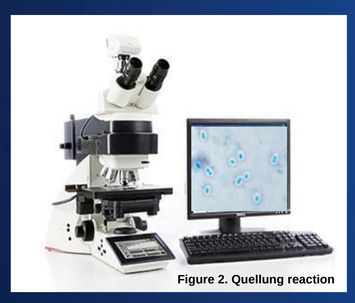

Figure 2. Quellung reaction

Table 1. Sequences of the 55 couple of primers included in the pneumoccocal serotyping multiplex-PCR

Serotype/Serogroup	Amplicon size (bp)	Primer Forward	Sequence primer forward (5'-3')	5'-Label	Primer Reverse	Sequence primer reverse (5'-3')
14	84	Csp-14-fw	CCGTCTTTTTGTATGGTGCTATG	HEX	Csp-14-rv	TGAACAGCCAATCCATCAATCAG
8	94	Csp-8-fw	ATTAGCTGCATACGCAAGAACC	NED	Csp-8-rv	GTTAATCCCCAAATAGGATCCC
7B,7C,24B,24F,40	103	Csp-72440-fw	AGACAATCTGCAGATATGATTCC	FAM	Csp-72440-rv	TATAAAAGTACCGTCCGCATTCC
9L,9N	111	Csp-9LV-fw	CACTGTTGGCTATGTTAGCCTC	HEX	Csp-9LV-rv	CTCTCCACGTGGCCAATATAC
21	122	Csp-21-fw	TTATGCTGGTTTAAATATCGCTCC	NED	Csp-21-rv	TAACAAATATGCCAAAACGTAGCC
34	130	Csp-34-fw	TGTAAGAGGAGATTATTTTCACCC	FAM	Csp-34-rv	GTCACAATAAAAACTGTACCTCC
25A,25F,38	140	Csp-25AF38-fw	CAACACAATGTCTTATTGCAGCC	HEX	Csp-25AF38-rv	AACGCACCCCAAAATAACTTTCC
10A,10B,10C,10F	151	Csp-10-fw	CRATGAGGCTATATGTTGGAATAG	NED	Csp-10-rv	GTATTGAACYCATAGATAACAGAG
43	160	Csp-43-fw	GGAATAGTTTAGGATTTGTACACC	FAM	Csp-43-rv	TAGAGTCTGCTAACTGTAATATCC
9A, 9V	172	Csp-9AV-fw	GATCAATGGCAACTATATTTACCC	HEX	Csp-9AV-rv	GATTCACTGTCTGACTTTGAACC
22A,22F	181	Csp-22-fw	ACGTATAGGACGTTTCTCAATCC	NED	Csp-22-rv	ATCCCGAAACCAAATTGCTATCC
3	191	Csp-3-fw	AGAAATGCTATCCGCGTTGGG	FAM	Csp-3-rv	TTGTCACGAGATTACGCTCAGG
15B,15C	201	Csp-15BC-fw	CGGATGATTGTAGCGTTTTATCC	HEX	Csp-15BC-rv	ACTGTAGATTGTGTTCTGATTCC
6A,6B,6C,6D	211	Csp-6-fw	GAAGTAGAAAATCGTGTAAGTGG	NED	Csp-6-rv	TCCAACAACTAACCTTATAAGGG
18A,18B,18C,18F	220	Csp-18-fw	AGTCTTACTAGACGTAATGAACC	FAM	Csp-18-rv	AAGATAAATTGACTAAGTCCTCCC
36	230	Csp-36-fw	TTCCGGATCTATTCAATTTCCCC	HEX	Csp-36-rv	CAATAACAGCCTCCGTTTTACC
27	240	Csp-27-fw	AATGCCGACGATTAATGCAGCC	NED	Csp-27-rv	CTAGCCATGCTGGATATTTCCC
29	251	Csp-29-fw	TGTGGCAAAAATTTCTTTAGCGG	FAM	Csp-29-rv	ATATACCCAGTAAACAGACAAGG
23F	260	Csp-23F-fw	TTCACAAGTGATAGTGAACTTGG	HEX	Csp-23F-rv	TATTAGCTTTATCGGTAAGGTGG
19A	269	Csp-19A-fw	ATTGGAGTAGCTGAGGTTTTTGG	NED	Csp-19A-rv	TATCCAATTTAAAACCAGCACGG
33C	278	Csp-33C-fw	CGGCAGGTATAAGTATTATCGG	FAM	Csp-33C-rv	CCTACACCTCTTATAAACGTTGG
20	292	Csp-20-fw	TATTGTTCCGAAAAAAGAGTGGG	HEX	Csp-20-rv	TACTCAAAGATTGTGTGGTACGG
31	301	Csp-31-fw	TTTCAAGGATATGATAGTGGTGG	NED	Csp-31-rv	TAGCATTACAGATGTCACTAAGG
23A	314	Csp-23A-fw	CTAGGTTCGTATCTCTTTGCGG	FAM	Csp-23A-rv	TACCAAATGGGTAATGGAGGGG
13	328	Csp-13-fw	TTAACAGGTAGATTACGACTTGG	HEX	Csp-13-rv	ATATCCCAAAAACAAAATCGCTGG
19B, 19C	338	Csp-19BC-fw	TCAGTACAGAATAGATGGAACACC	NED	Csp-19BC-rv	CCCAGTATCTAAATCCTAATCCC
11A,11D,11F	348	Csp-11ADF-fw	GGACATGTTCAGGTGATTTCCC	FAM	Csp-11ADF-rv	TGCGCCAAATTTGGTATCGACC
32A,32F	357	Csp-32-fw	CTTACAATGAGACGCTATTTTCC	HEX	Csp-32-rv	GTTATTACCATTGAATTCGTTCCC
35A,35C,42	367	Csp-35AC42-fw	TCGTTCACCTACTTTATTAATGCC	NED	Csp-35AC42-rv	AATAATTCCTAATACCATCTGCCC
35F,47F	375	Csp-35F47F-fw	TTGCTACAGTTTTGATGTATCTCC	FAM	Csp-35F47F-rv	AGAATCCGTTTCATCATACAGCC
2	383	Csp-2-fw	GTTCAATATTTCTCCACTACACC	HEX	Csp-2-rv	CTAAGAGTTCCAATACGTTGACC
11B,11C	392	Csp11BC-fw	TAGAAATCGCAAGATAGCCTTCC	NED	Csp11BC-rv	CTGATTATGAGCATAGTTGATCC
17F	402	Csp-17F-fw	TCTTGTCAAATACATACTTACCCC	FAM	Csp-17F-rv	GTAGTCTCGCATTTCTATCATCC
41A,41F	413	Csp-41-fw	AGTTACTGGCCCTTTCTTATTCC	HEX	Csp-41-rv	AGGTAAAAAGTCATATCCATTCCC
47A	423	Csp-47A-fw	AATACATTGTACGTCTTTAACCCC	NED	Csp-47A-rv	CGAAGAATTAAACCCACATAACC
4	433	Csp-4-fw	ATTCAGAGGCAGCTAGTTCAGG	FAM	Csp-4-rv	CAGAAGCTACTGTTAGGCTGG
5	443	Csp-5-fw	TTATCTATTTTATCGCAGACTCCC	HEX	Csp-5-rv	CTGCCGATAAAAAGATAGATGCC
33A,33F,37	453	Csp-33AF37-fw	ATGTTAGATTAGATGGTTTGCTGG	NED	Csp-33AF37-rv	ATTCAACACATAAACCGTTGGGG
45	463	Csp-45-fw	CAGATTGGTTTTCACATCACTCC	FAM	Csp-45-rv	ACATAACACGACTTTTAGTGACC
6C,6D	473	Csp-6CD-fw	GTCGTGTAAAGTAGTATACAATCC	HEX	Csp-6CD-rv	ATAATCCTCTGGATTATCCCACCC
35B	483	Csp-35B-fw	CATTAGTGTTGCTATGTTGTTCC	NED	Csp-35B-rv	GATTAGATAAATAAATACGCCCCC
7A,7F	491	Csp-7AF-fw	TTGACTGCAAGTGTTTCAATGGG	FAM	Csp-7AF-rv	AAAGCACAAAATATTGGAACGAGG
16A	501	Csp-16A-fw	GTTATGATAATGGTAACGCCTCC	HEX	Csp-16A-rv	CAGCCAATAAGTCATATACGCC
19F	523	Csp-19F-fw	TGTTCTTAGTAATGGATATACGGG	NED	Csp-19F-rv	AAAACTTCACCAGGATCTAATGG
2	383	Csp-2-fw	GTTCAATATTTCTCCACTACACC	HEX	Csp-2-rv	CTAAGAGTTCCAATACGTTGACC
11B,11C	392	Csp11BC-fw	TAGAAATCGCAAGATAGCCTTCC	NED	Csp11BC-rv	CTGATTATGAGCATAGTTGATCC
17F	402	Csp-17F-fw	TCTTGTCAAATACATACTTACCCC	FAM	Csp-17F-rv	GTAGTCTCGCATTTCTATCATCC
41A,41F	413	Csp-41-fw	AGTTACTGGCCCTTTCTTATTCC	HEX	Csp-41-rv	AGGTAAAAAGTCATATCCATTCCC
47A	423	Csp-47A-fw	AATACATTGTACGTCTTTAACCCC	NED	Csp-47A-rv	CGAAGAATTAAACCCACATAACC
7A,7F	491	Csp-7AF-fw	TTGACTGCAAGTGTTTCAATGGG	FAM	Csp-7AF-rv	AAAGCACAAAATATTGGAACGAGG
48	532	Csp-48-fw	CCCTTTGATAGCTATAGTATCGG	FAM	Csp-48-rv	CCCTGGAATAGAAGTTTTCTAGG
39	544	Csp-39-fw	TATGAGGTATCATTTAGCAGGGG	HEX	Csp-39-rv	ATCATCGAAATGGCAACTAAAGG
1	555	Csp-1-fw	TTTGCTAGATGGTGAGTTTGTATC	NED	Csp-1-rv	TTTAGAAGCTGCATTGTACTACTC
24A,24B,24F	569	Csp-24-fw	TCATGCTTATGTTATGTGTTACGG	FAM	Csp-24-rv	GTGAGAGCTATATTTAGAACATGG
28A,28F	578	Csp-28-fw	AGGTAGACTACCAATTTCAATTCC	HEX	Csp-28-rv	TACACCTGCTAATATCAATGTTCC
15A,15F	598	Csp-15AF-fw	ATGAGAGGAAGATATACTGGG	NED	Csp-15AF-rv	AAATAAGTTGTCCCATAGGAAGG
16F	606	Csp-16F-fw	ACTGCTTGCATATTAGCTTTATGG	FAM	Csp-16F-rv	TGATAGAGTGACAGAACAATTGG
17A	615	Csp-17A-fw	TTTACCCAAGAATGGTTTCTAGG	HEX	Csp-17A-rv	AGCATAACAGTTTGCGCTATTGG
12A,12B,12F,44,46	627	Csp-124446-fw	CCTTTCTGATTCGTCCAGTTCC	NED	Csp-124446-rv	AGTTGAACCAACTCCCCATCC
33B,33D	640	Csp-33BD-fw	ACAACAGCAATGTTGTTGTTACTC	FAM	Csp-33BD-rv	GAGAAGTAAGAGTTTTGTCATCC
23B	650	Csp-23B-fw	TTGCATATGGATTTAATGGTGGG	HEX	Csp-23B-rv	ACCATTGCTGATAGAAGTAGAGG

Figure 3. *Streptococcus pneumoniae* serotyping based on PCR hibridation/assay

Results

In 7 (5.6%) isolates the *cps*A gene was not detected, nor by the multiplex PCR containing the *cpsA*, *lytA*, and *ply* genes nor by the PCR/hybridization assay. Of them, 2 were non-encapsulated pneumococci (Omni Serum negative), nevertheless 5 were capsulated with positive reaction for primer-set for serotypes 25A,25F,38. The serotyping results perfectly matched with that of the Quellung reaction.

There were four serotype 38 (3 causing respiratory infections and one otitis) and one 25A (invasive pneumonia).

The usage of more than one technique for *S. pneumoniae* serotyping avoid us the erroneous characterization of these 5 (4%) isolates.

Conclusions

- Emerging serotypes with deficiencies in the *cpsA* gene after PCV13 introduction, as serotypes 25A and 38, could be not detected or considered non-capsulated if only molecular techniques targeting this gene are used.
- PCR/hybridization assay as well as multiplex PCR with fluorescent labelled primers followed by amplicon analysis showed to be excellent methodologies for pneumococcal detection and serotyping.

References:

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