

# A *Streptococcus pneumoniae* serotype 19F *wzy* variant in Venezuela and pneumococcal vaccine cross-protection



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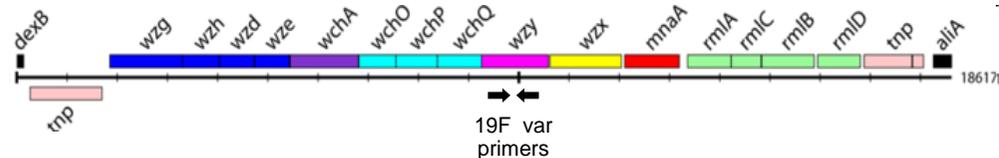
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## INTRODUCTION

The emerging of new or variant pneumococcal serotypes has become a major concern for the surveillance of invasive pneumococcal disease (IPD) and vaccine protection. New serotypes or variants of existing serotypes have an uncertain vaccine cross protection and may be the cause of IPD, escaping vaccine protection. The capsular polysaccharide biosynthesis locus (*cps locus*) containing the genes responsible for capsule synthesis is the location for most of the genetic rearrangements that lead to the arise of novel serotypes. In Venezuela, a commonly occurring *S. pneumoniae* serotype 19F *wzy* variant, typed as 19F with the Quellung reaction, but not detected using PCR with published primer sets, has been found as a colonizer of the nasopharynx of Warao Amerindians and as the etiological agent of meningitis in the capital Caracas. Here we characterize these strains genetically and investigate if the conjugate vaccine protects against this variant strain.

## MATERIALS AND METHODS

Because the primers for the PCR based serotyping of serogroup 19 target the *wzy* gene, the *wzy* gen of the variant serotype 19F was sequenced. With the available sequence information, primers were designed targeting the variant *wzy* gen for the detection with PCR. Multilocus sequence typing (MLST) was performed to determine the Sequence Type (ST) of a representative group of the variant strains. Mice were immunized to determine with a pneumococcal opsonophagocytic killing assay (OPKA) pneumococcal vaccine cross-protection for these 19F variant strains.



## REFERENCES

1.- Menezes AP, Reis JN, Ternes YM, Andrade AL, Pimenta FC, Carvalho Mda G, Beall B. Update of pneumococcal PCR serotyping assay for detection of a commonly occurring type 19F *wzy* variant in Brazil. J Clin Microbiol. 2013;51(7):2470-1.

## RESULTS

Sequence information of the *wzy* gen of the variant strain revealed that the variant *wzy* gen shares 99.9% identity with a *wzy* 19F variant isolated in Brazil from carriage and invasive sources [1]. All our variant 19F strains had a positive PCR signal of  $\approx$  607 bp with the newly designed primer set. 85% of the 19F strains recovered from the eastern part of the country carried the *wzy* variant gen. In this Amerindian population, the 19F variant was shared among the members of 8 families, with 3 mothers sharing the variant serotype with their child. PCR serotyping found two other variant 19F strains isolated from pneumococcal meningitis in Caracas.

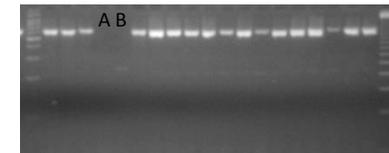


Fig.1 PCR with primers targeting the *wzy* gene of the 19F variant strains. Strains A and B did not carry the altered gene.

MLST of 10 of these pneumococcal variant strains, isolated on different time points, showed that 8 strains were of Sequence Type ST391. Another two strains were ST3260 and SLV1801.

The differences in the polysaccharide structures of 19A and 19F can be attributed to the *wzy* polymerase gene and is associated with the putative linkages catalysed between D-Glcp and  $\alpha$ -L-Rhap ( $\alpha$ -1,2 in type 19F and  $\alpha$ -1,3 in type 19A). Given the lack of cross-protection between 19F and 19A and the key role of the *wzy* gen in those serotypes, we investigated vaccine cross-protection for our variant 19F strains. As demonstrated by OPKA, a cross-reactive immune response was elicited against the 19F variant strains in mice immunized with a 19F strain.

## CONCLUSIONS

A 19F serotype variant previously reported in Brazil has been isolated in Venezuela as the etiological agent of 2 cases of meningitis in Caracas and as a frequent colonizer in the Warao Amerindians in the east of the country. A Primer set was designed for a PCR based detecting of this 19F variant strains. OPKA results indicated that pneumococcal vaccination will most probably protect against this variant 19F strain.