

Detection of the Hmw adhesins in clinical *Haemophilus influenzae* isolates from bacteraemic patients and association with biofilm formation

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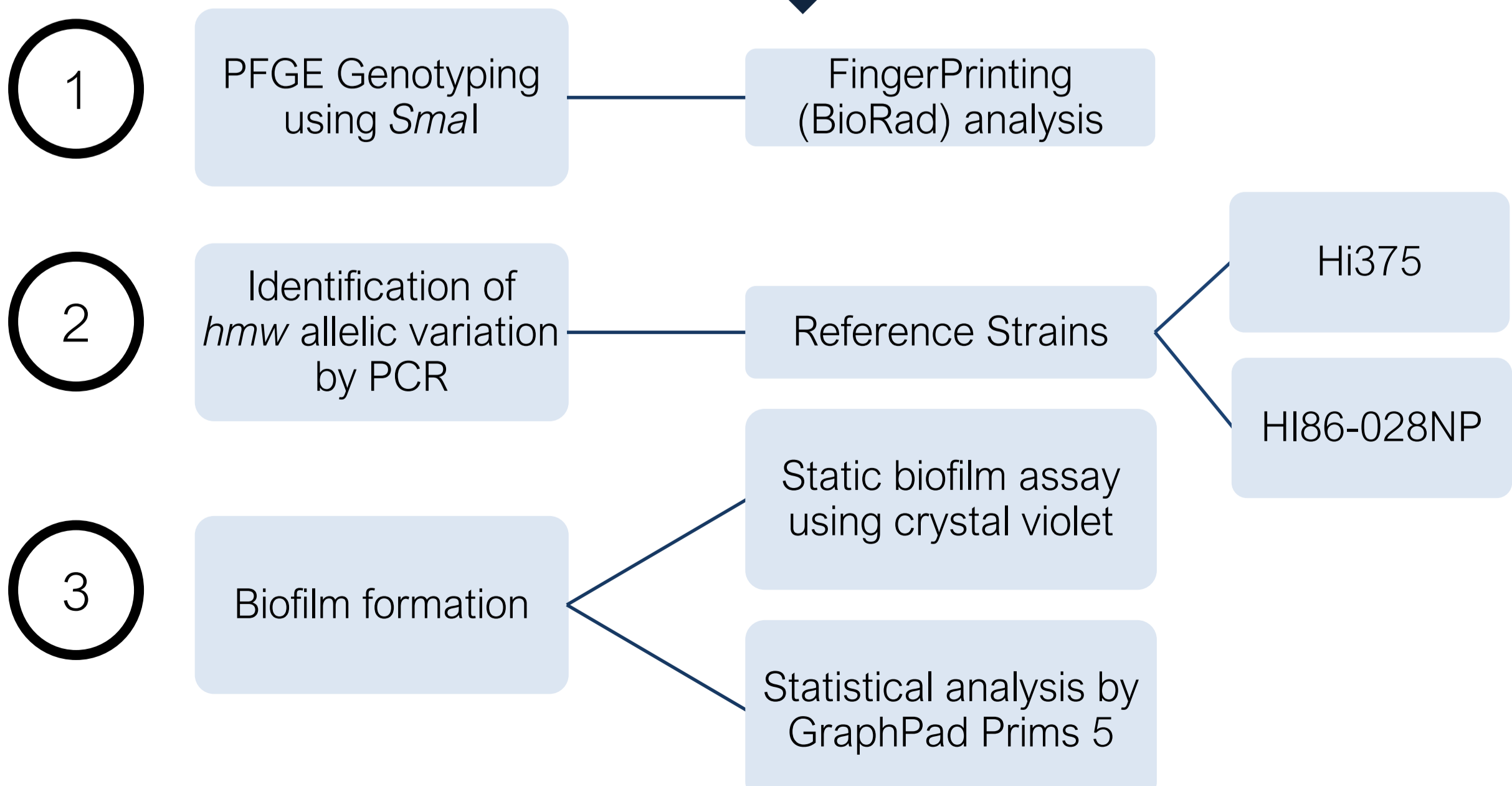
INTRODUCCION

Non-typeable *Haemophilus influenzae* (NTHi) forms part of the normal nasopharyngeal microbiota in humans, but it is also an opportunistic pathogen causing respiratory infections and bacteraemia. Recently, high molecular weight (HMW1) proteins have been identified as a key factor for cell invasion, a feature implicated in persistence during chronic infection (Mell, JC et al. (2016). PLoS Pathogens 12: e1005576). Our aims were to identify the different allelic variants of the HMW adhesin and, given the characteristics of these surface proteins on bacterial adhesion capacity, the second objective was to check if their presence could be related to biofilm formation.

METHODS

A collection of 89 strains isolated from patients with bacteraemia from Spain and Portugal in the 2013-2014 period were used in this study. Strains were genotyped by PFGE (*Sma*I) and analyzed with the FingerPrinting software (BioRad). The allelic variants of the *hmw* gene (Hi375 and Hi86-028NP) encoding the high molecular weight adhesins Hmw1/Hmw2 were identified by PCR amplification. Biofilm formation was performed in a static biofilm assay with crystal violet staining. Statistical analysis was performed using the GraphPad Prism 5 software.

Strains selection				
Country		Period		Source
Spain	Portugal	2013	2014	Sterile places



RESULTS

Forty-eight NTHi isolates (54%) were positive for the *hmw* gene. Only the allelic variants of the Hi375 strain could be identified, among them, one strain (1/48, 2%) had *hmw-1A*, 33 (69%) had *hmw-2A* and 14 (29%) had both allelic variants, *hmw-1A* and *hmw-2A*. Biofilm formation showed great diversity among the studied strains with OD₅₇₀ values ranging between 0.06 and 1.4. Forty-three strains (48.3%) were classified as high biofilm formers and the remaining 46 strains (51.7%) were low biofilm formers.

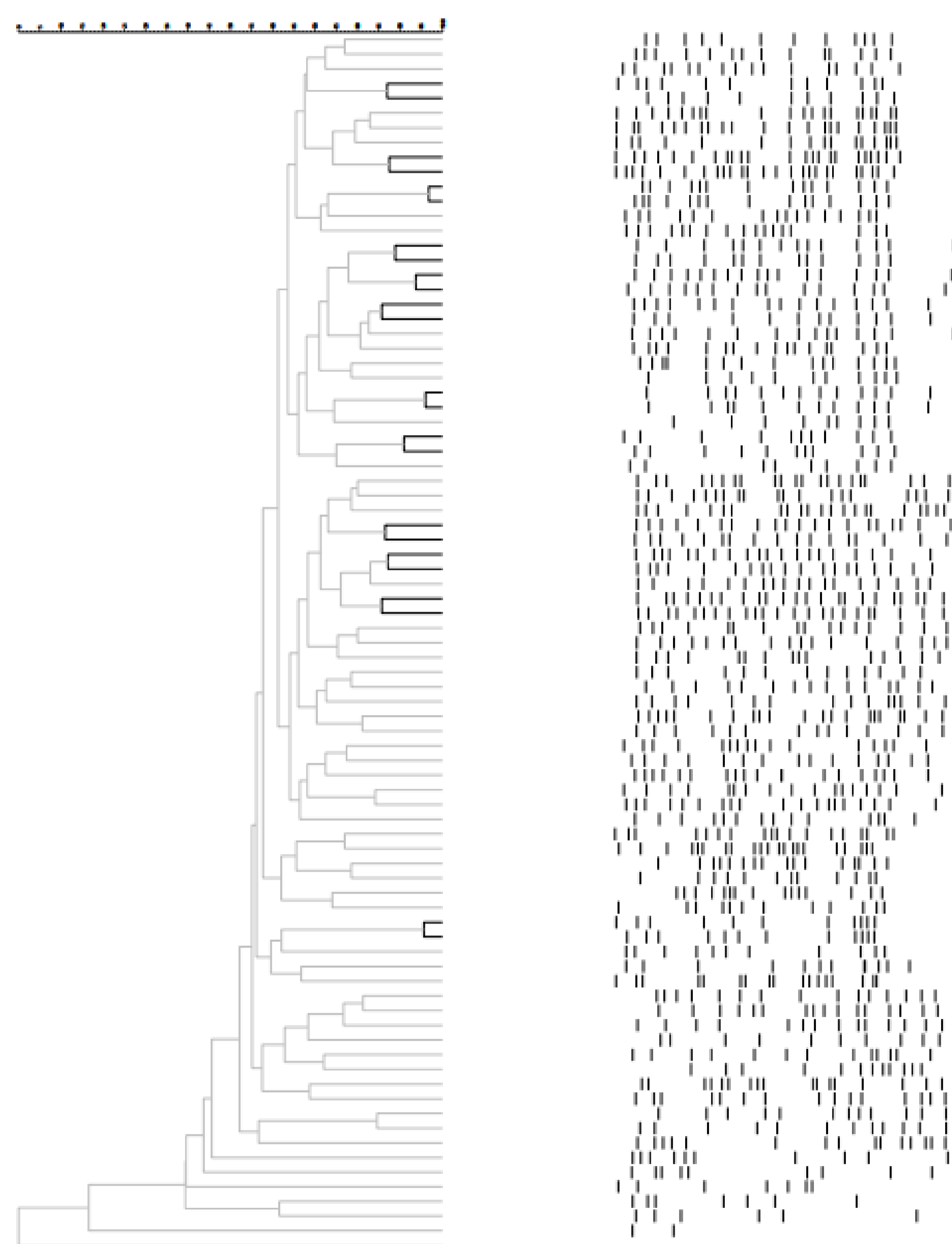


Figure 1. Dendrogram. Distribution of 89 NTHi isolates analysed by PFGE using *Sma*I

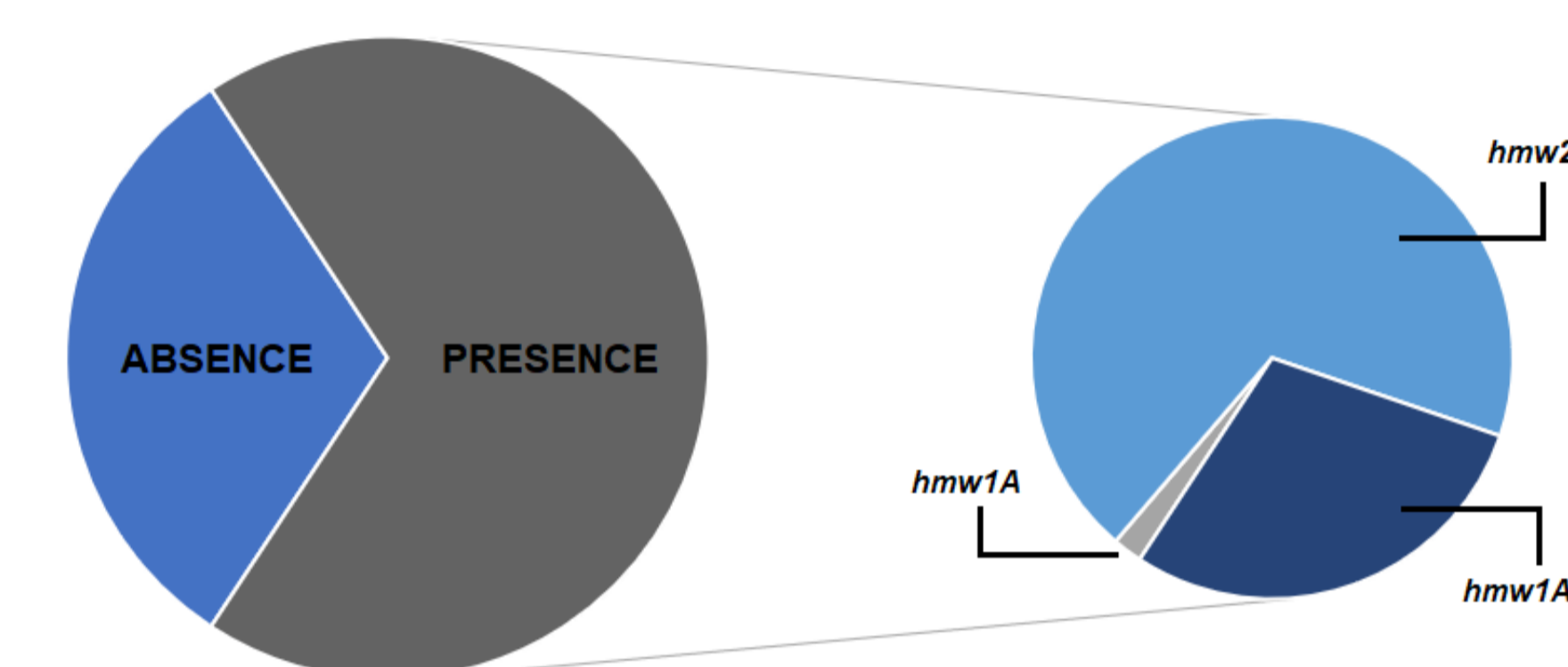


Figure 2. PCR results for *hmw* genes amplification. A total of 41 (46%) of the strains did not have any gen. At least one gene was presented in 56% of the strains. Of them, 2% had *hmw1A*, 69% had *hmw2A* and 29% had *hmw1A/2A*.

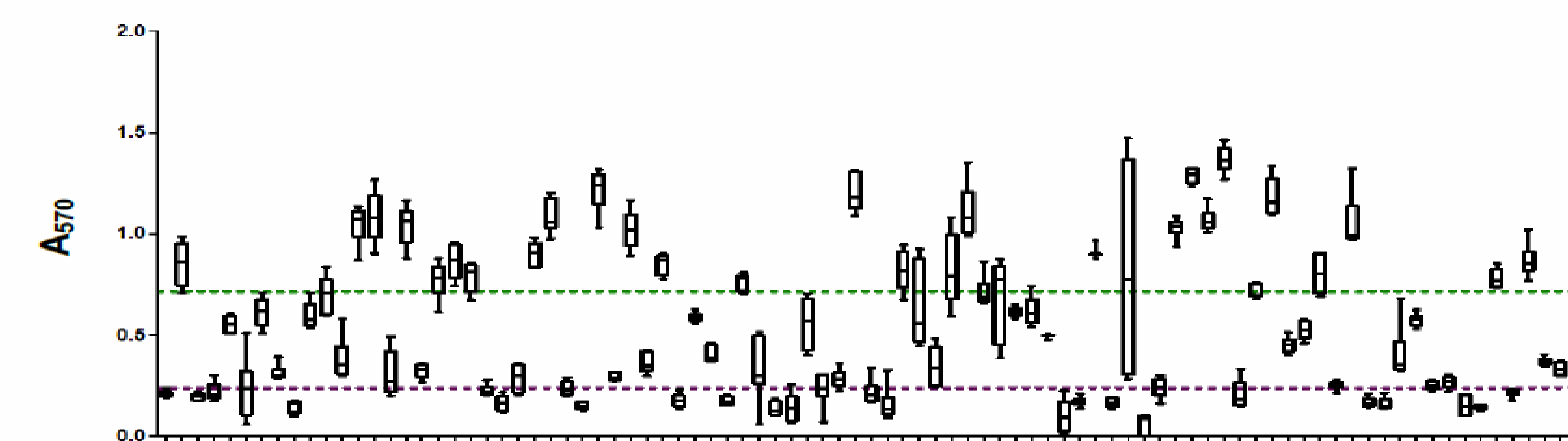


Figure 3. Variation of biofilm formation for each analysed strain.

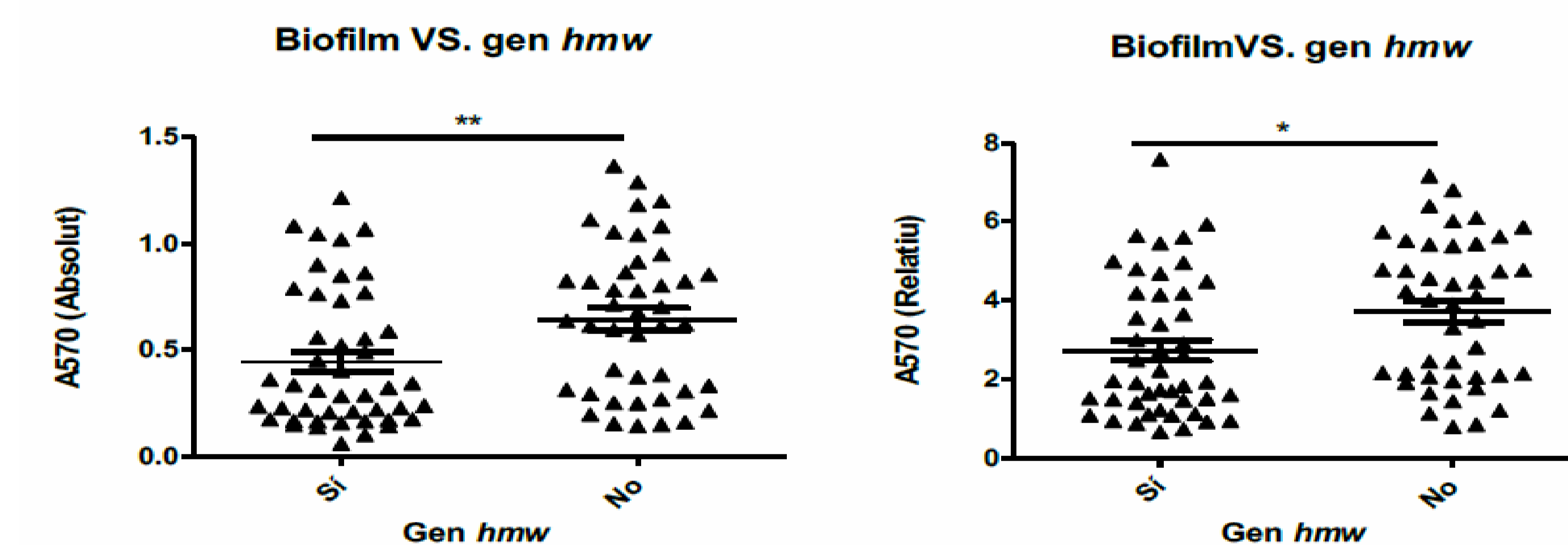


Figure 4. Variation of biofilm formation based on the presence (one or both alleles) or absence of *hmw* gene. (A) shows absolute values and (B) shows relative values of biofilm formation. *P<0,05; **P<0,01.

CONCLUSIONS

An inverse relationship was found between the presence of *hmw* genes and *in vitro* biofilm formation. The invasive NTHi clinical isolates presented high genetic diversity by PFGE, with no strain clustering observed linked to the presence of *hmw* genes or to biofilm formation. The allelic variants of the *H. influenzae* strain 375, especially the *hmw-2A* gene, were more commonly found among invasive NTHi clinical isolates, which despite having an important role on intracellular invasion, were not linked to *in vitro* biofilm formation.