Suppression of mismatch repair in cells of pathogenic *Neisseria* species enhances the antigenic variation of *pilE*

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1 Introduction

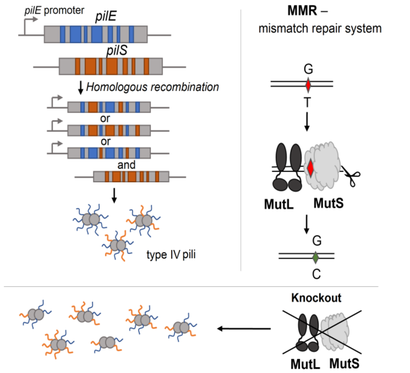

Fig. 1. Process of antigenic variation of PilE by homologous recombination (left panel); process of mismatch repair by MMR proteins — MutS and MutL (right panel); effect of knockout of mutS and mutL genes on the antigenic variation efficiency (bottom panel).

Pathogenic *Neisseria* species have an antigenic variation of pilin, the main component of type IV pili, which are the most important virulence factor.

It has been shown that knockout of genes of key proteins of the MMR system (MutS and MutL) increase the antigenic variation of *pilE*.

Possible ways of regulation:

• Inactivation of MMR leads to accumulation of mutations.

• MMR proteins suppress homologous recombination.

2 Aim

To find the correlation between *pilE* variability and complete or partial deletion of DNA regions encoding MutS and MutL proteins in the genomes of *N. gonorrhoeae*.

3 Analysis of *mutS* and *mutL*

The tools of the bacterial database PubMLST allow to get information about the alleles of *mutS* and *mutL* and their prevalence among different strains of *N. gonorrhoeae*.

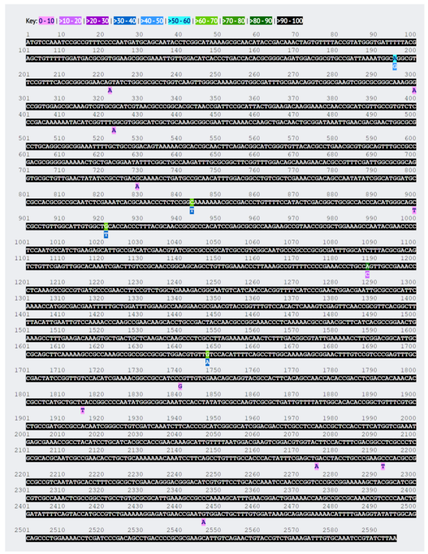

Fig. 3. Polymorphism map of the most common mutS alleles in N. gonorrhoeae.

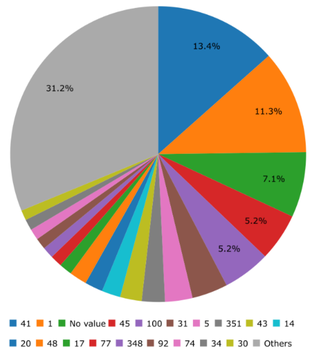

Fig. 2. Diagram of the distribution of mutS alleles among N. gonorrhoeae isolates.

MutS recognizes the DNA mismatch and MutL makes a single-stranded break in the damaged DNA, initiating the repair process.

Both MutS (Figures 2, 3) and MutL (data not shown) are conservative within the *N. gonorrhoeae* population.

Nucleotide polymorphic sites occures with low frequency and don't influence on structure or functions of proteins.

4 Gene Presence Analysis

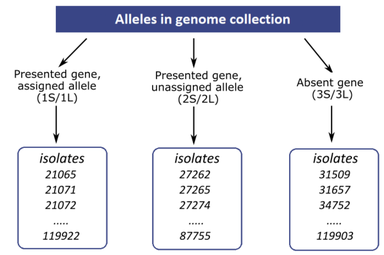

Fig. 4. Dividing of N.gonorrhoeae isolates genomes with mutS/mutL-presence or absence.

Based on the *mutS/mutL* gene presence, all isolates were divided into three groups (Fig. 4). At this stage, the PubMLST *Dataset* plugin was used, which allows obtaining the allele ID for each isolate. Strains with unassigned alleles or without genes have no allele ID in database. The *Gene presence* plugin was used to determine the presence of the genes in isolates.

5 Analysis of *pilE*

• Due to limitation of the PubMLST database, we used BLAST (The Basic Local Alignment Search Tool) and the *pilE* sequence of *N. gonorrhoeae* strain MS11 as a consensus to retrieve the gene from all isolates. The processes of collecting sequences and building alignments were separated.

Position	Variant	Frequency
1	A	15.28%
1	T	0.23%
1	-	84.49%
2	T	15.51%
2	-	84.49%
3	-	84.49%
3	G	15.51%
4	A	15.28%
4	C	0.23%
4	-	84.49%
5	A	15.28%
5	C	0.23%

Table 1. Example of Python script output.

• Alignments in Jalview and ClustalO EMBL-EBI were built for each group.

• Alignments were analyzed using a Python script.

• During the analysis, the frequencies of different nucleotide variants in all positions were determined for each alignment (Table 1).

• We determined the average variability of *pilE* polymorphic sites in each group of *N. gonorrhoeae* isolates as the proportion of isolates whose *pilE* gene contains a substitution at the polymorphic position (Fig. 5).

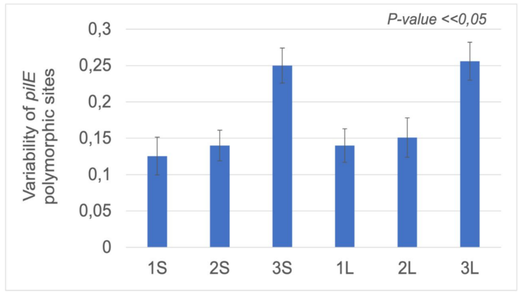

Fig. 5. The average variability of pilE polymorphic sites in each group of N. gonorrhoeae isolates.

• According to statistical analysis, Student's t-test revealed a statistically significant difference in mean variability between groups 1S/L and 3S/L, 2S/L and 3S/L but not between 1S/L and 2S/L.

• It was shown that the absence of the *mutS* or *mutL* genes in the 3S and 3L groups of *N. gonorrhoeae* isolates increased the mutation frequency in *pilE*.

6 Conclusion

Using bioinformatics tools, we analyzed the genomes of *N. gonorrhoeae* isolates deposited in the PubMLST database. It has been established for the first time that the loss of *mutS* or *mutL* genes leads to an increase in *pilE* variability in the cells of pathogen isolates. This may be used by bacterium to avoid a human immune response. Regulation of MMR process in cells of pathogenic *Neisseria* spp. is a potential approach to the treatment of gonorrhea.