Comparative whole genome analysis of Staphylococcus saprophyticus reveals mutation of uro-adherence factor A (UafA) gene in non-urinary strains and a clonal expansion in urinary tract infections in Marseille.

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Introduction

- Staphylococcus saprophyticus is one of the leading cause of Urinary Tract Infections (UTIs) diseases in young ages, especially in female, accounting for up to 40% in this population.
- Recently, in Marseille, we have observed an abnormal increase of reported cases from January 2002 to December 2004, suspecting a possible outbreak or spread of clustered strains. Virulence factors of S. saprophyticus involved in UTIs includes adherence to urothelial cells by means of a surface-associated protein, lipase, lipothiocic acid, hemagglutinins that bind to fibronectin, uropepsin, and production of extracellular slime. A whole genome sequencing of S. saprophyticus subsp. Saprophyticus 13503 isolated from a young women urine has revealed a single orf predictable as a cell-wall-anchored protein and it showed a positive hemagglutination and adherence to human bladder cells[1].
- We aim to conduct an epidemiological investigation within the suspected outbreak period to confirm the increasing of UTIs due to Staphylococcus saprophyticus in Marseille as well as performing a real time genomic to decipher any specific genomic features that may explained this phenomenon.

Materials and Methods

- We performed a retrospective statistical analysis of S. saprophyticus involved in UTIs diseases using the R software.
- A S. saprophyticus/G744 whole genome was sequenced, assembled, annotated and analyzed using various bioinformatics tools.

Results

Over the same period, 39,095 patients have experienced E. coli/UTI. Throughout the study period, S. saprophyticus/E. coli UTIs ratios increased 3.9 fold from 0.84 in 2002 to 3.28 in 2014, with an annual estimated trend of the ratio of 6.10^(-7) (p-value < 10^-3) (Figure 3). We observed a significant increasing of the number of S. saprophyticus UTIs (from 2 138 vs 18 in 2002, to 4 599 vs 151 in 2014, p-value < 10^-7).

Figure 5: Graphs show distribution and evolution of various components of pan-genome: 1- total genes evolution of the Pan-genome 2-core or conserved genes evolution 3- unique genes evolution 4- number of blastp hit with different percentage identity 5- association between new genes and unique genes in the pan-genome.

Figure 6: Heatmap derived from an average nucleotide identity matrix calculated with Gbaculars of S. saprophyticus isolated from various samples showing low average nucleotide identity among clinical isolates.

Figure 7: Heatmap of the previous pan genome matrix, with dendrogram sorting genomes and sequences.

Figure 8: Genome sub-region visualization showing codon stop mutations that fragmentated ano adherence protein into two fragment within two clinical strains and genome size variation.

Table 1: Sex and Age distribution of patient from whom isolates were collected for hemagglutination test.

<table>
<thead>
<tr>
<th>Age</th>
<th>Male</th>
<th>Nice</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;12</td>
<td>6</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>1-18</td>
<td>10</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>&gt;18</td>
<td>38</td>
<td>12</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>33</td>
<td>87</td>
</tr>
</tbody>
</table>

Conclusions

Our study confirmed clonal expansion of S. saprophyticus in a community UTIs outbreak. This clonal expansion was associated with positive hemagglutination enabling the bacteria to be more pathogenic. Negative hemagglutination correlates with mutation of UafA gene in non-clinical strains. Pan-genome analysis shows high plasticity, stable core genome and open pan-genome indicating possible evolution and emerging of uro-pathogen strains. No new virulence genes have been found to be associated with uro-pathogenicity of S. saprophyticus, G744. Hemagglutination test can be used as surrogate test and a surveillance marker for detecting S. saprophyticus uro-pathogen strains in the lab as well as biological surveillance tools.

References

1. Kédjovi D. Mlaga; Codric abdr.Joan-Marc Rotman. Unité de recherche sur les maladies infectieuses et tropicales émergentes (URMITE) CNRS-BIOM U9236, Méditerranée Infection, Faculté de Médecine et de Pharmacie, Aix-Marseille Université, Marseille, France.