Rapid culture-independent diagnostics of infectious diseases by Oxford Nanopore genomic sequencing

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Background

Infectious diseases affect millions of people every year. Particular virulent and multi-drug resistant agents are increasingly responsible for infections with ever-expanding complexities. Therefore, it is critical to establish rapid, efficient and accurate diagnostic methods to apply the proper treatment as soon as possible

Purpose

Optimization and application of Oxford Nanopore long-range DNA sequencing, as a rapid and high-resolution Next- Generation Sequencing (NGS) method for identification of pathogens directly from clinical samples, and study of the potential application in routine clinical laboratory

Method

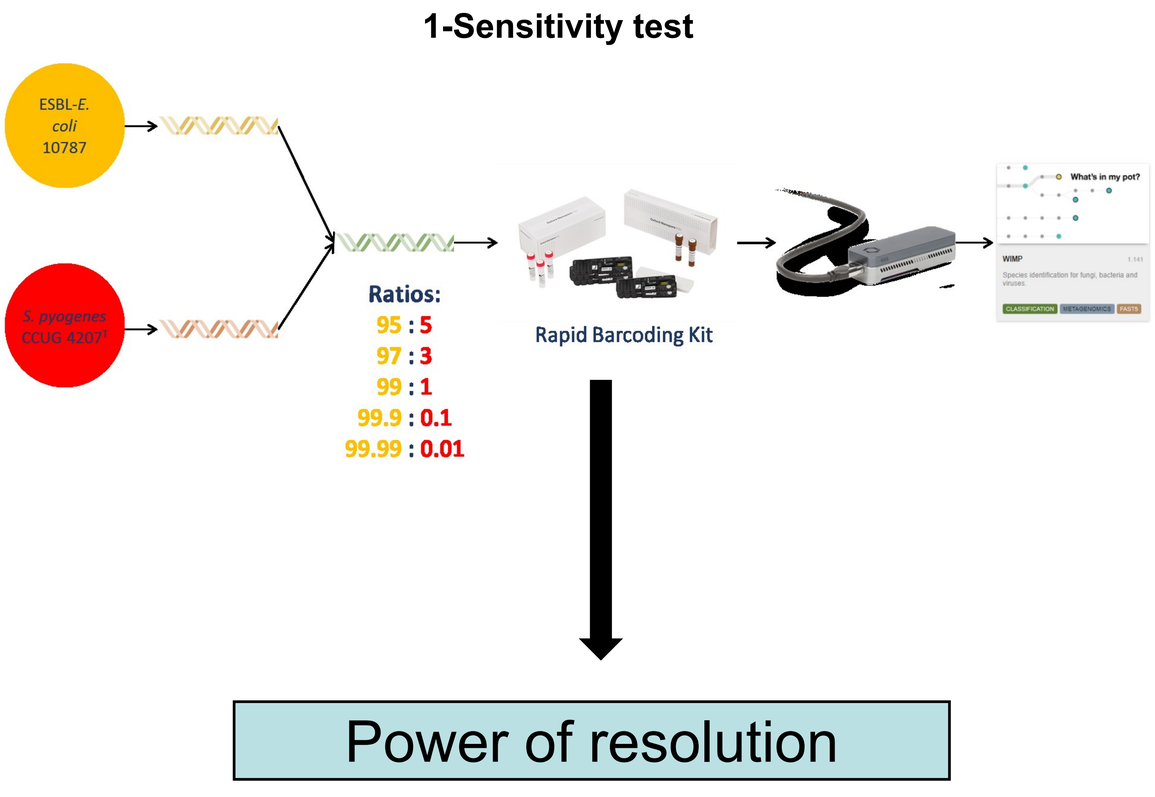

Figure 1. Analysis of the resolution of MinION using known mixtures. DNAs were mixed in different percentages to study the capacity of MinION of identify the two species even in low amounts of DNA.

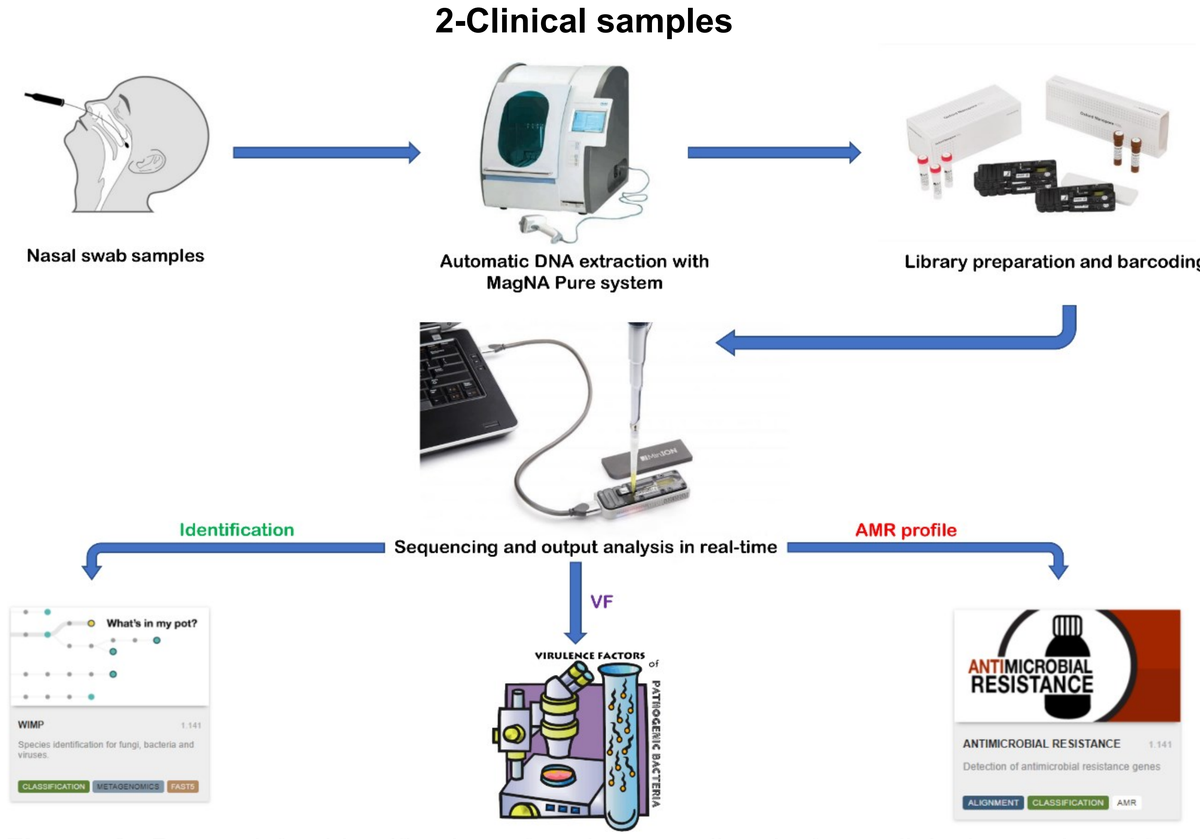

Figure 2. Protocol for identification of pathogens directly from clinical samples. After automatic DNA extraction, sequencing libraries are prepared and sequenced with MinION. The data is analized in real-time

Results

Mixed samples using *E. coli* and *S. pyogenes* DNAs showed that MinION is able to identify a microorganism even if there was only 1 out of 10.000 reads (Figure 3). Analysis of some clinical samples, already confirmed by the routine lab, ended with the detection of the pathogen in less than 7 h (counting the whole process) and less than 30 min after the sequencing itself was started (Figure 4).

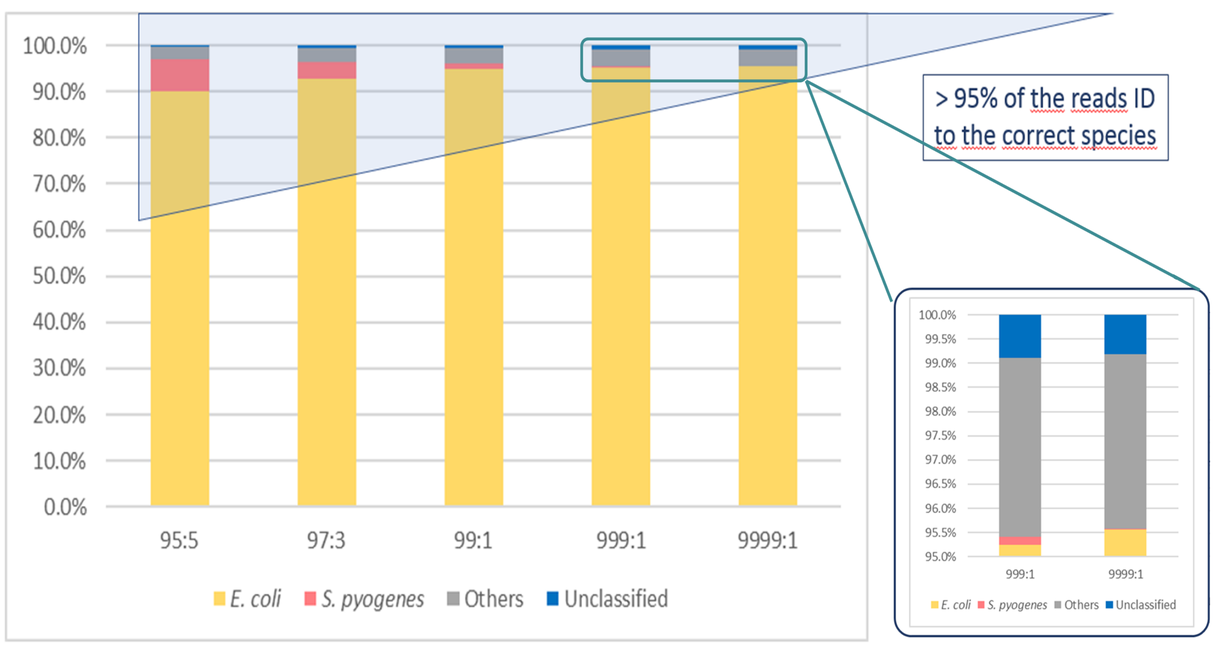

Figure 3. Identification of mixtures of DNA with two known species. *Streptococcus pyogenes* was identified even when there was only 0,01% of DNA in the sample.

Figure 3. Results of the analysis of two clinical samples. In 30 min of sequencing, reads identifying the correct pathogen were detected.

Conclusions

MinION has a high power of resolution which make it suitable to analyze complex samples. It is possible to detect pathogens in a fast and culture-independent way, even though more optimization work is needed

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