Antimicrobial resistance probability by nanopore-based metagenomics

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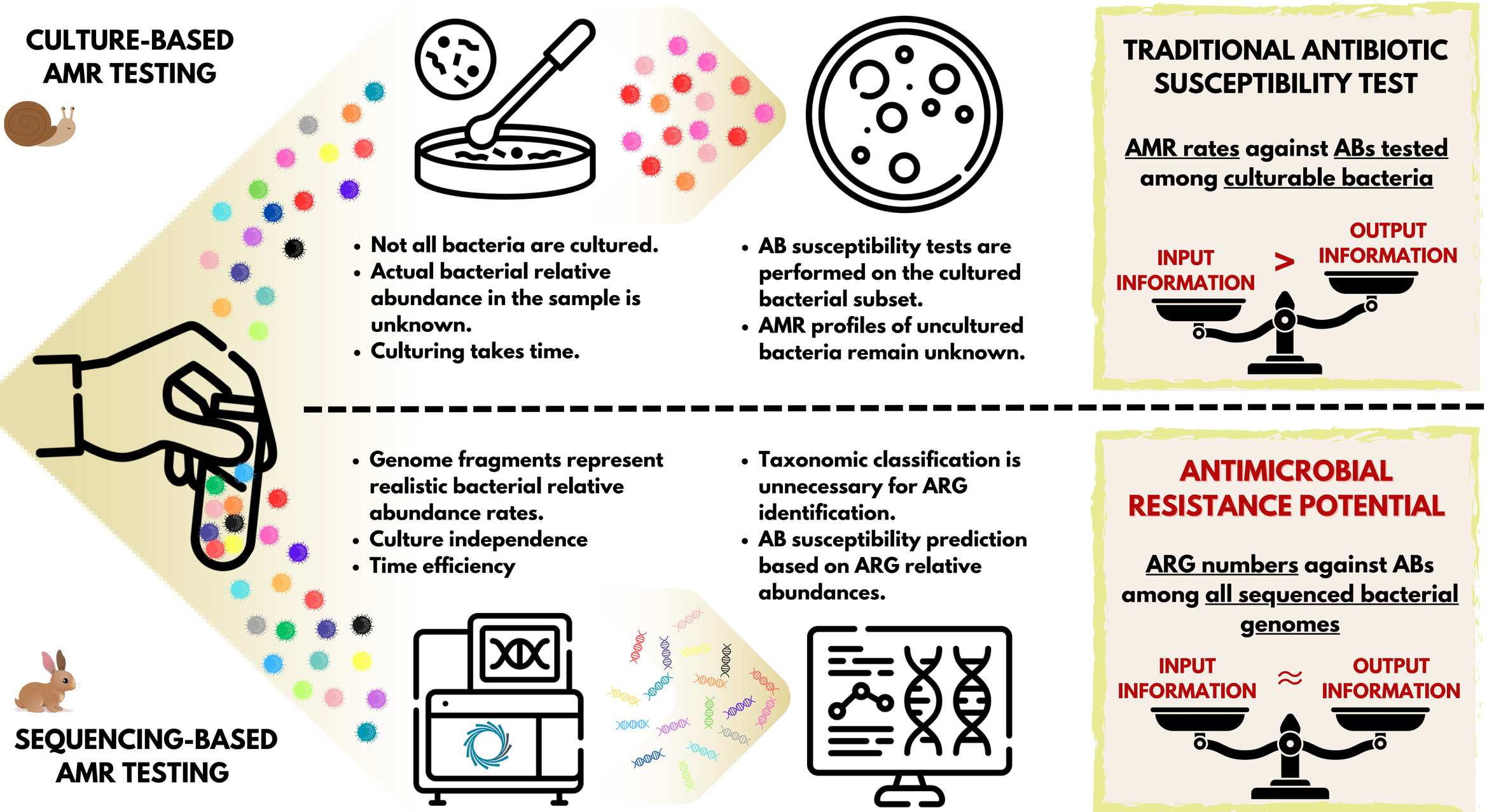


Figure 1. Summary of the potential concept of nanopore sequencing based rapid, taxonomic classification free antibiotic susceptibility analysis of metagenomic samples.

Introduction

To hinder the spread of antimicrobial resistance (AMR) and ensure the most proper, targeted treatment options, antibiotic prescriptions are often based on antibiotic susceptibility tests.

At the same time, multiple microorganisms are involved simultaneously at several infections. Routine culture conditions favor the growth of certain bacteria over others. The presence of bacterial species with special growth needs remains hidden and the relative abundance rates of taxa from the original sample are untraceable. Consequently, we only gain knowledge of the phenotypic antibiotic susceptibility of the culturable subset of bacteria, while these AMR rates do not necessarily represent the most abundant taxa in the original sample.

Furthermore, even the fastest growing bacterial species require 1-3 days to be cultured and tested, while slow-growing species remain undetected.

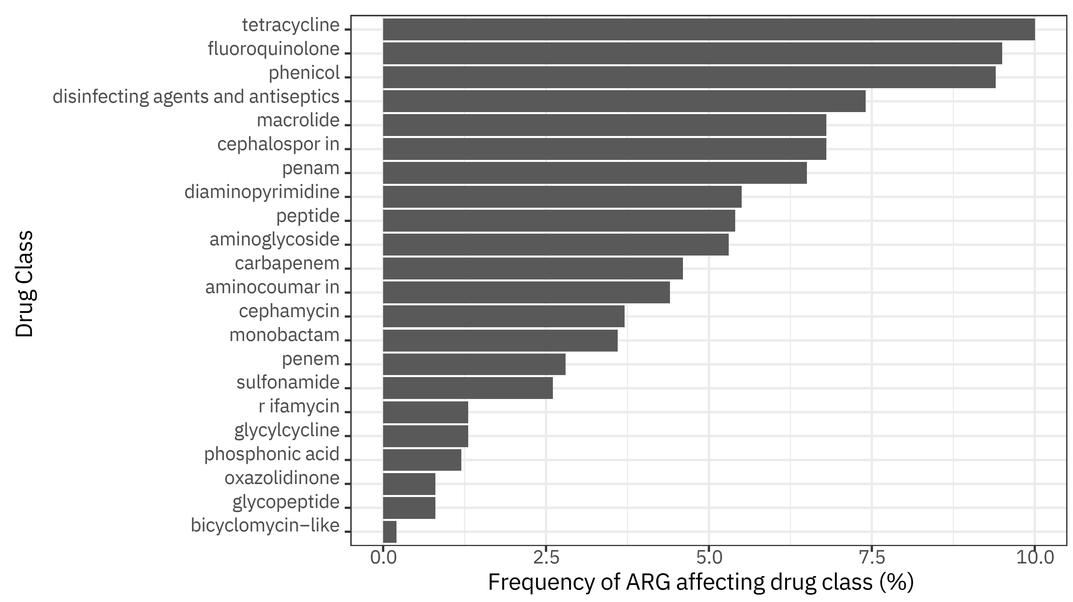
Objectives

Within this study, we aimed to demonstrate a possible future use of nanopore shotgun sequencing as a rapid characterization tool for antimicrobial susceptibility by metagenomic samples with complex microbiomes.

Methods

To demonstrate the concept, swab samples from a canine chronic, severe otitis externa case were collected. The samples were sent for routine diagnostic microbiology testing and sequenced using the Rapid Barcoding Kit 24 V14 (SQK-RBK114.24) from Oxford Nanopore Technologies (ONT). The sequencing was implemented with a MinION Mk1C sequencer using an R10.4.1 flow cell from ONT. Bioinformatic analysis was performed following the pipeline of Tóth et al.[1] using the Comprehensive Antibiotic Resistance Database (CARD, v.3.2.9) and the Resistance Gene Identifier (RGI, v6.0.3) with Diamond.

Results



By the traditional routine test, multi-resistant *Pseudomonas aeruginosa* was identified and, if locally administered, ceftazidime, gentamicin, tobramycin, ciprofloxacin, marbofloxacin and polymyxin B were indicated to be potentially effective. Based on the panbacterial antimicrobial resistance gene (ARG) numbers, peptides (polymyxin B) and aminogylcosides (tobramycin, gentamicin) are favorable, along with further, non-tested drug classes.

Conclusions

Nanopore sequencing-based ARG number detection (AMR Potential) can become one of the members of the everyday clinical diagnostic toolset by mixed microbial samples due to its preservativeness for genomic relative abundance rates and its time efficiency.

References

1. A.G. Tóth et. al. (2023) First animal source metagenome assembly of Lawsonella clevelandensis from canine external otitis. bioRxiv 2023.12.17.572052; doi: https://doi.org/10.1101/2023.12.17.572052

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