Real-time genomics for rapid clinical pathogen identification and resistance prediction in bloodstream infections

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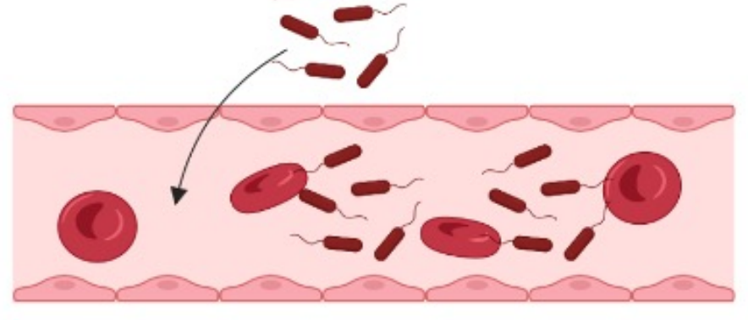
Introduction

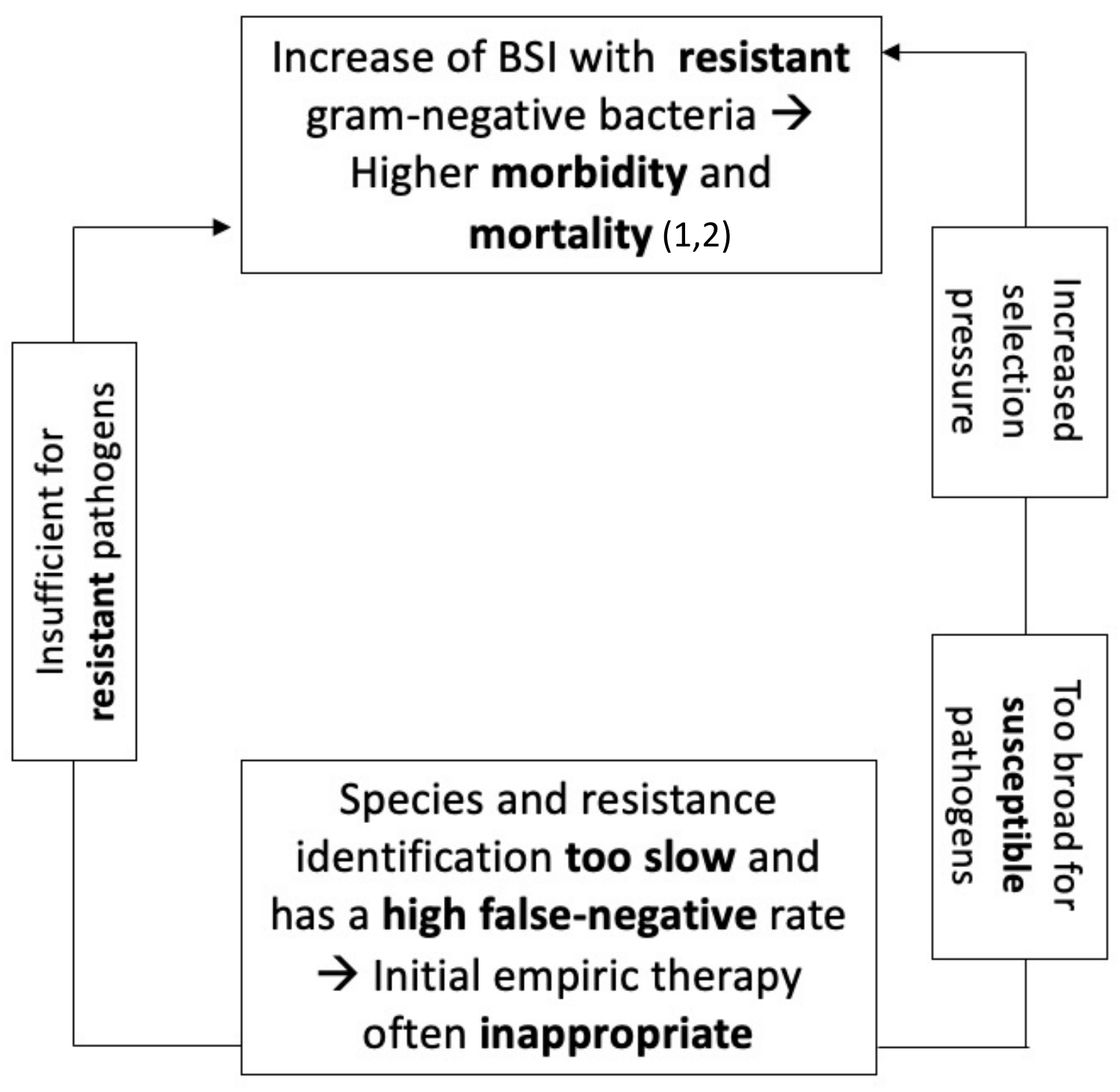
Starting point



Bloodstream infection (BSI): Presence of viable microorganisms in the bloodstream. Major cause of morbidity and mortality in a hospital setting

Rapid administration of appropriate antimicrobial therapy as main prognostic factor

Challenges



Increase of BSI with **resistant** gram-negative bacteria → Higher **morbidity** and **mortality** (1,2)

Increased selection pressure

Too broad for **susceptible** pathogens

Species and resistance identification **too slow** and has a **high false-negative** rate → Initial empiric therapy often **inappropriate**

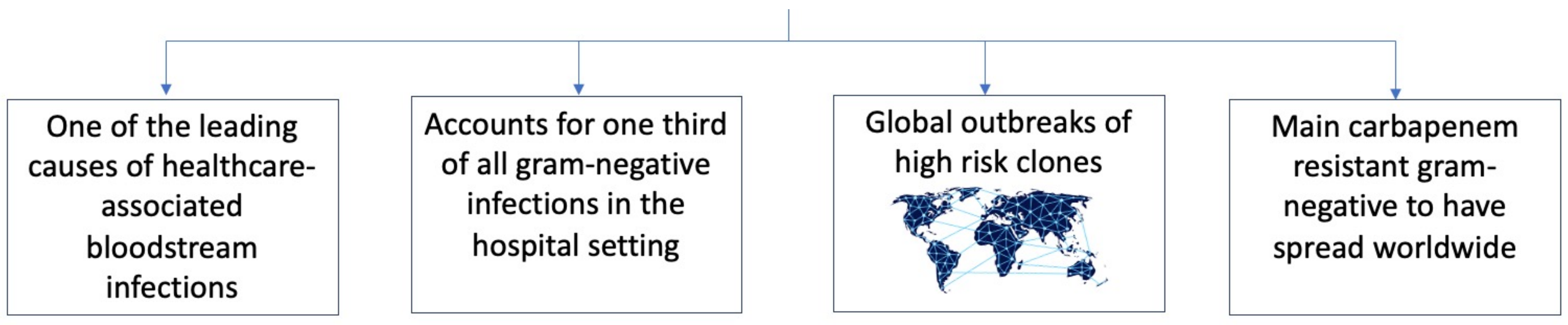
Insufficient for **resistant** pathogens

Background

Why focus on gram-negative bacteria?

- Gram-negatives as WHO pathogens of concern due to their growing resistance rates
  - Natural inhabitants of the GI-tract of humans and animals
    →High exposure to antibiotics
    →High potential for transmission

- In particular, *Klebsiella pneumoniae* harbours an abundance of resistance and virulence genes (3,4)



One of the leading causes of healthcare-associated bloodstream infections

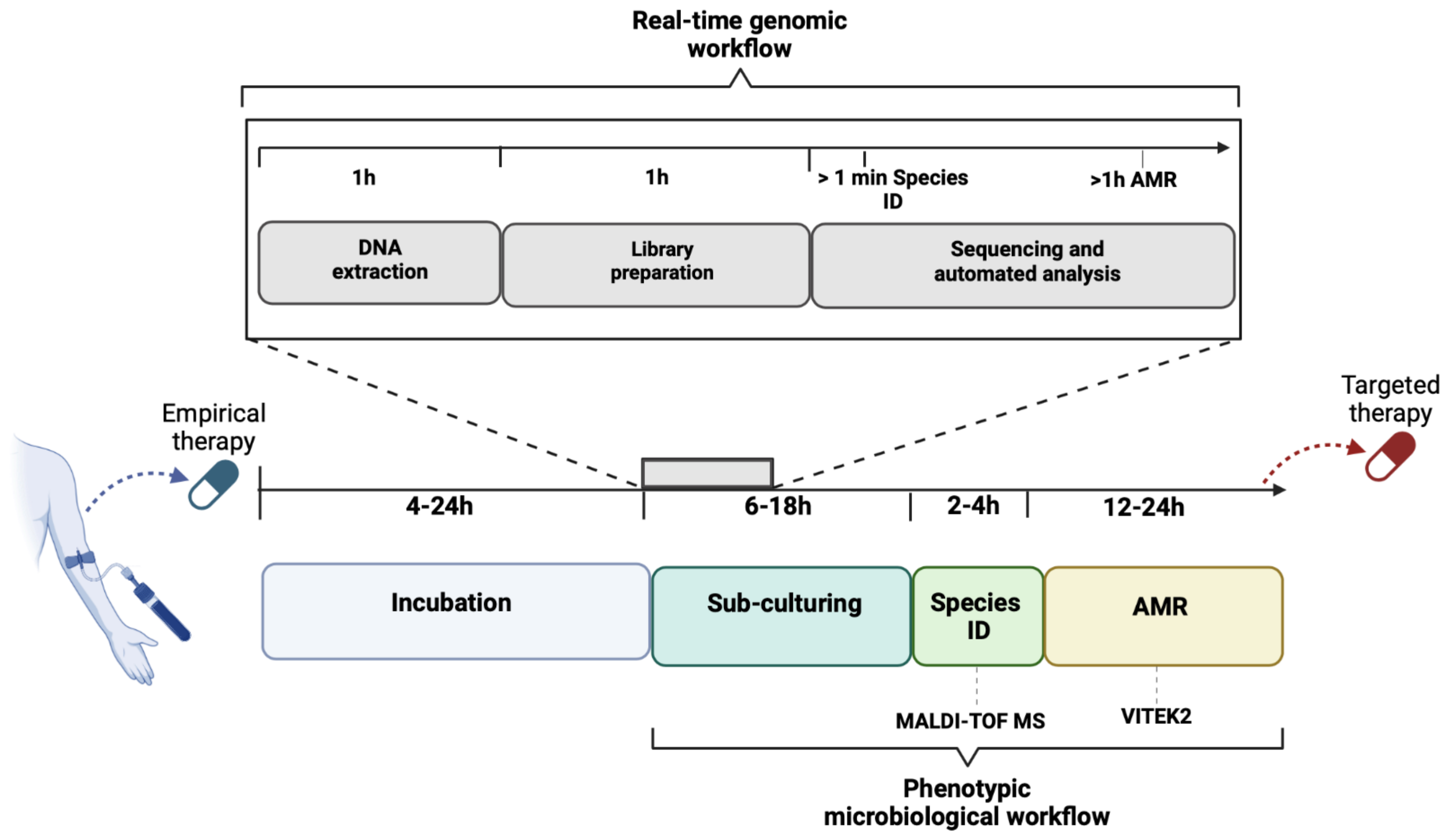
Accounts for one third of all gram-negative infections in the hospital setting

Global outbreaks of high risk clones

Main carbapenem resistant gram-negative to have spread worldwide

Why use real-time genomics for bloodstream infections?

- Genomic information to understand evolution and transmission (5)
- Metagenomics to assess the entirety of microorganisms in the bloodstream
- Rapid identification of resistance and virulence patterns (5)
- Reduction of turnover time (6)



Study population and data

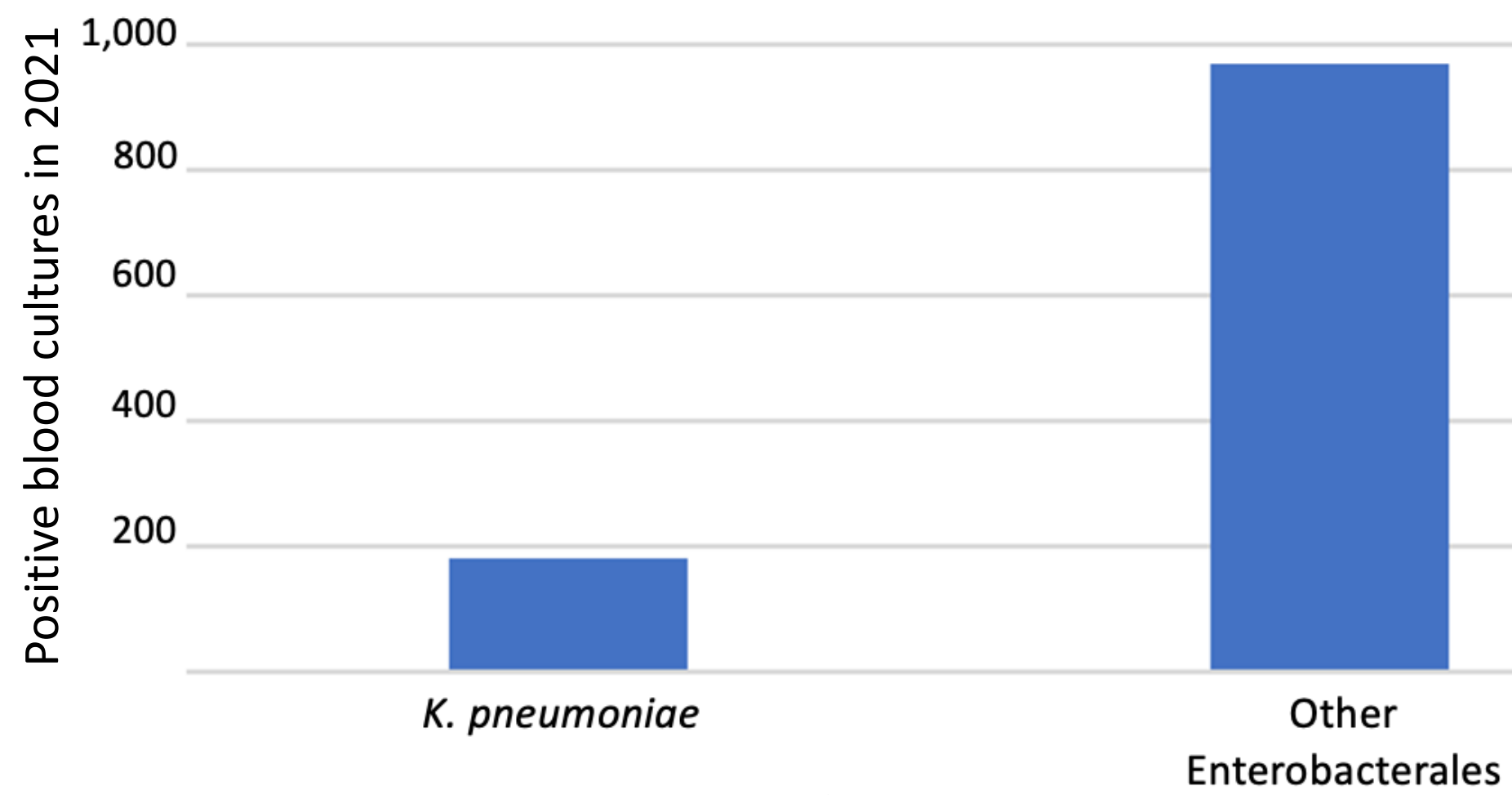
Study population

- ICU patients at TUM Rechts der Isar with confirmed gram-negative bloodstream infection

Data

- Phenotypic bacterial identification and antimicrobial susceptibility testing
- Patient data: Clinical outcome, laboratory results

- Sequencing data:
  - Stored samples: *Klebsiella pneumoniae* & other *Enterobacterales*, contaminant bacteria
  - Gram-negative blood cultures: Expected sample size 100-150



Overview statistics of TUM Rechts der Isar

Outlook

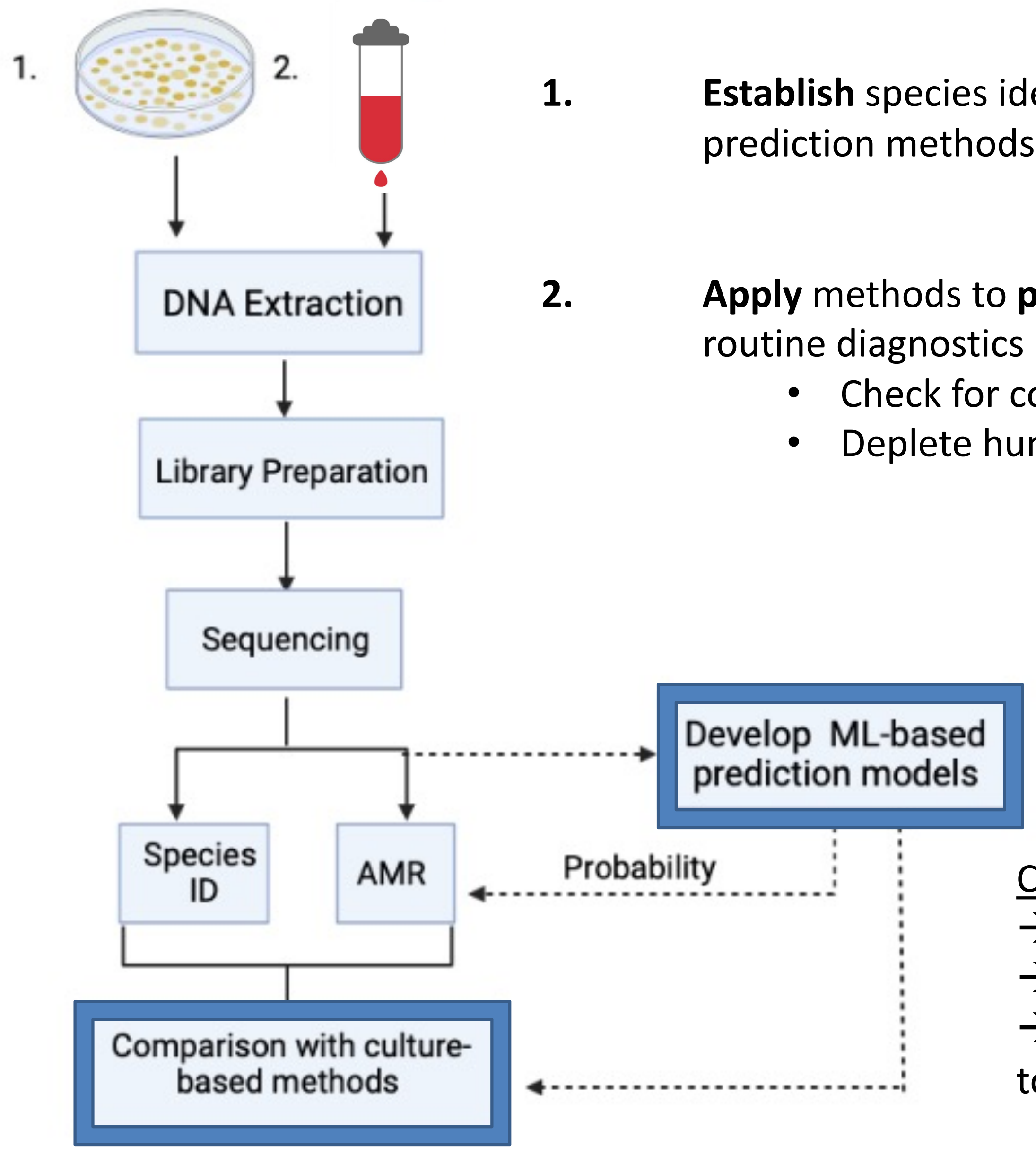
- Optimize computational analysis to create metagenome assemblies and resistance predictions from positive blood cultures (using R10.4.1)
- Intervention modelling on the impact of reduced turnover time on patient outcome
- Analysis of *K pneumoniae* samples from neonatal bloodstream infection cases in Harare, Zimbabwe → Compare genomic population structure and resistance patterns

Aims

1. Accurately identify and describe resistance patterns of gram-negative bacteria, in particular, ***K. pneumoniae***, causing bloodstream infection from stored samples at the university hospital TUM Rechts der Isar in Munich, Germany.

2. Provide a **fast** and **accurate** real-time metagenomics approach to detect gram-negative bloodstream infections from positive blood cultures of ICU patients at TUM Rechts der Isar in Munich, Germany.

Study plan



1. **Establish** species identification and resistance prediction methods on **stored samples**
2. **Apply** methods to **positive blood cultures** from routine diagnostics
   - Check for co-infection
   - Deplete human DNA/contaminants

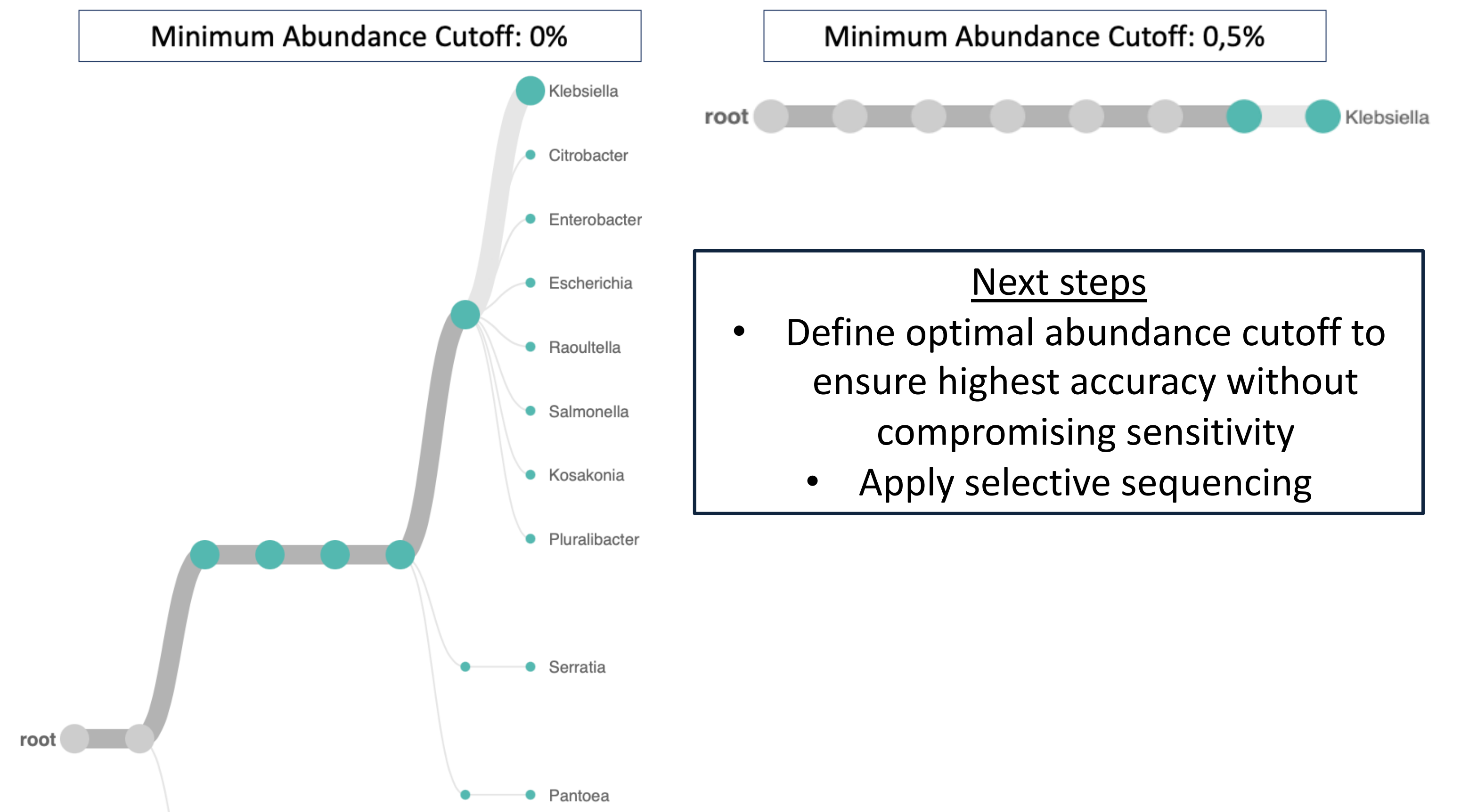
Compare and assess
→ Species identification
→ Resistance prediction
→ Time to result
to the culture-based approach

Preliminary Results

1. **Sequencing of a stored *K pneumoniae* isolate**

Parameter	R 9.4.1 SQK-RBK004 72h sequencing run
Sequencing depth	50
Number of reads	216.509
Read length N50	3.849 bp
Total bases	426.301.831

2. Species identification



Results generated using WIMP v3.2.1 ONT analysis tool based on CENTRIFUGE

Next steps
- Define optimal abundance cutoff to ensure highest accuracy without compromising sensitivity
- Apply selective sequencing

3. Resistance prediction

Resistance genes	Average accuracy	Alignments
oqxAB	92,9%	135
blaSHV-94	95,1%	12
blaSHV-172	94,8%	6
blaSHV-143	95,6%	5

Reduced fluorquinolone susceptibility

Ampicillin and Piperacillin R

Resistance prediction using Epi2me on the CARD database

Next steps
- Evaluate different accuracy cut-offs and resistance prediction models
- Develop *Enterobacterales*-specific model for fast and accurate resistance prediction

Antibiotics	AST
Ampicillin	R
Piperacillin	R
Ampicillin-Sulbactam	S
Piperacillin-Tazobactam	S
Cefotaxim	S
Ceftriaxon	S
Ceftazidim	S
Meropenem	S
Ertapenem	S
Cotrimoxazol	S
Ciprofloxacin	S
ESBL	-

Phenotypic resistance results (Vitek2)

References

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