

Streamlining Pathogen Surveillance: Evaluating NGS as a Rapid Alternative to Phenotypic Serotyping and AMR Testing

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Background and Aim

- Serotyping and antimicrobial resistance (AMR) testing** are critical components of infectious disease surveillance, outbreak investigation, and vaccine development.
- Conventional phenotypic methods are often **time-consuming, labor-intensive and limited in scalability**.
- Next generation sequencing (NGS)-based bioinformatics tools offers a promising alternative for **streamlined serotype and AMR characterization**.
- We aim to assess NGS-based AMR and serotype prediction bioinformatics tools using **diverse, multi-species in-house dataset**, with conventional phenotypic methods as the reference standard.

Method

Over 300 clinical isolates of *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, and *Salmonella enterica* were included in this study. All isolates underwent phenotypic testing and whole-genome sequencing to generate three comprehensive in-house datasets. We evaluated a range of publicly available AMR and serotyping bioinformatics tools using Illumina and Nanopore reads and assembly, with predictions compared to phenotypic results (**Fig.1**). Tools' accuracy, speed and generalizability were assessed.

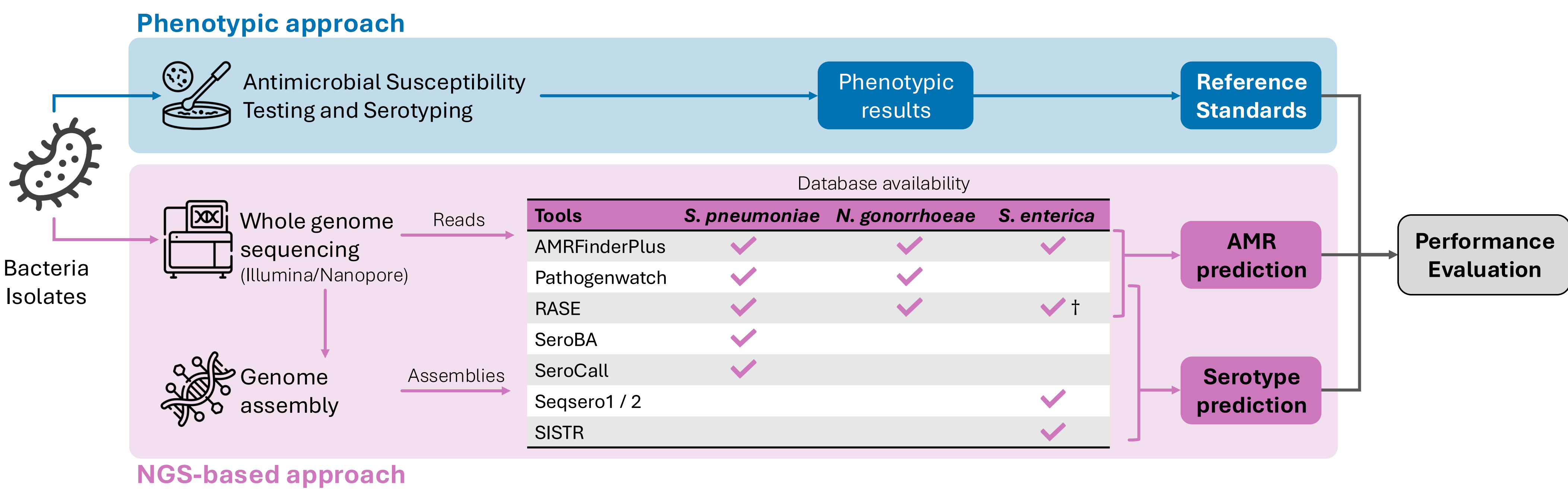


Fig.1: Workflow for evaluating AMR and serotyping bioinformatics tools. †Custom *Salmonella enterica* database was constructed.

Results

1. NGS-based approach contributes significant advantages over conventional phenotypic approach (Table.1)

Table.1: Comparison between conventional phenotypic approach and NGS-based approach of serotyping and antimicrobial testing.

| Feature | Phenotypic Approach | NGS-based Approach | NGS Implication |
|-----------------|---------------------------------------|--------------------------------------|---|
| Typeability | Low | High | Ensures more isolates are characterized |
| Resolution | Low | High | Provides strain-level resolution |
| Turnaround Time | Growth-dependent | 1-2 days | Facilitate faster and timely public health responses |
| Logistics | Requires extensive antisera and drugs | Streamlined workflow and consumables | Simplifies lab workflows, making surveillance more scalable |
| Susceptibility | Gold standard but growth-dependent | Investigative | Rapidly predicts resistance profiles for early guidance and track resistance genes surveillance |

2. High accuracy and expanded capability in serotype tools

- 93% concordance** with phenotypic results in *S. pneumoniae* dataset using Illumina paired-end reads (**Fig.2A**).
- Successfully predicted **12 out of 13 (92%)** *S. pneumoniae* that were non-typable by phenotypic method.
- Observed **similar predictive performance** in *S. enterica* using genome assemblies (**Fig.2B-C**).

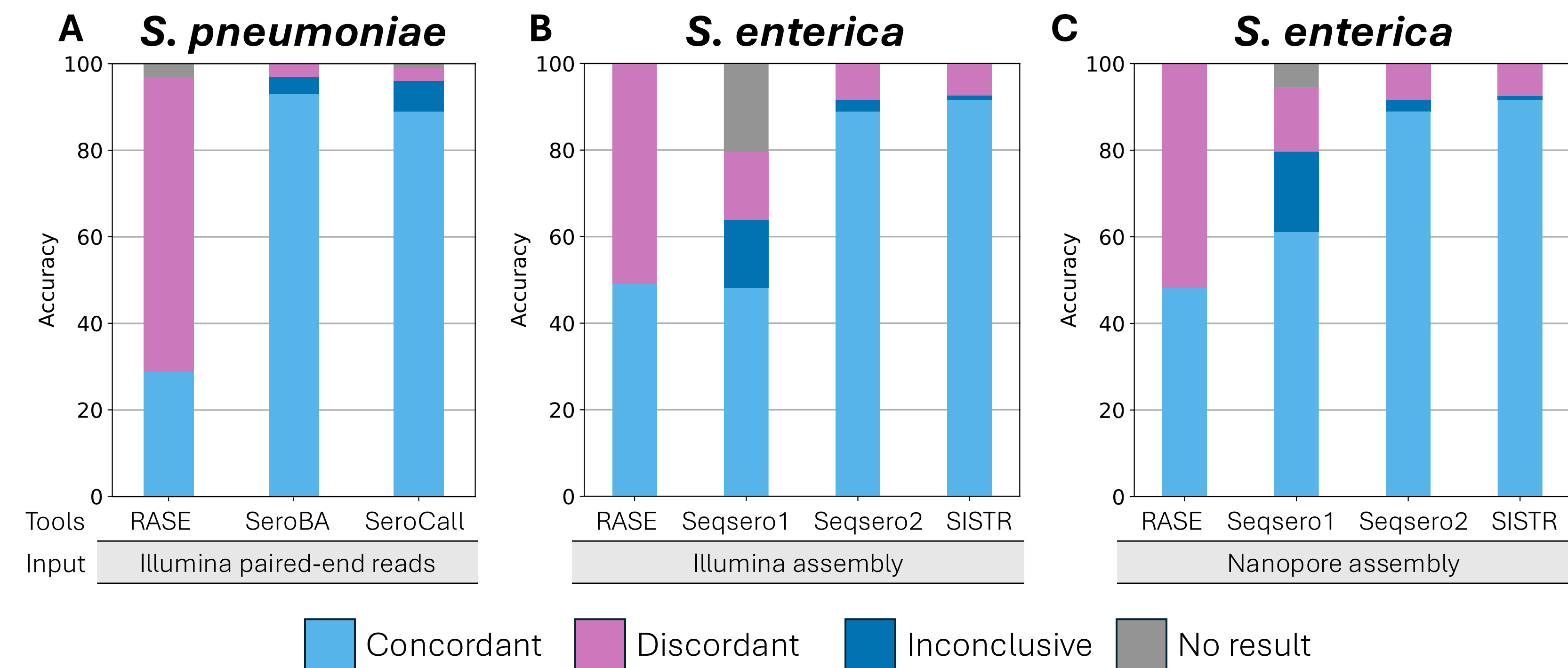


Fig.2: (A) Percent concordance with phenotypic results for *Streptococcus pneumoniae* tools (n=119) using Illumina paired-end reads. (B) Percent concordance for *Salmonella enterica* tools (n=112) compared to phenotypic results using Illumina and (C) Nanopore assemblies.

■ **Concordant** = Reference serotype matches prediction; ■ **Discordant** = Reference serotype does not match the prediction; ■ **Inconclusive** = Tools output 2 serotype predictions where one is correct; ■ **No result** = no serotype prediction output.

3. AMR tools offer high predictive performance

NGS-based AMR predictive tools achieved F1 score of **0.87-0.92** across all three species (**Fig.3**).

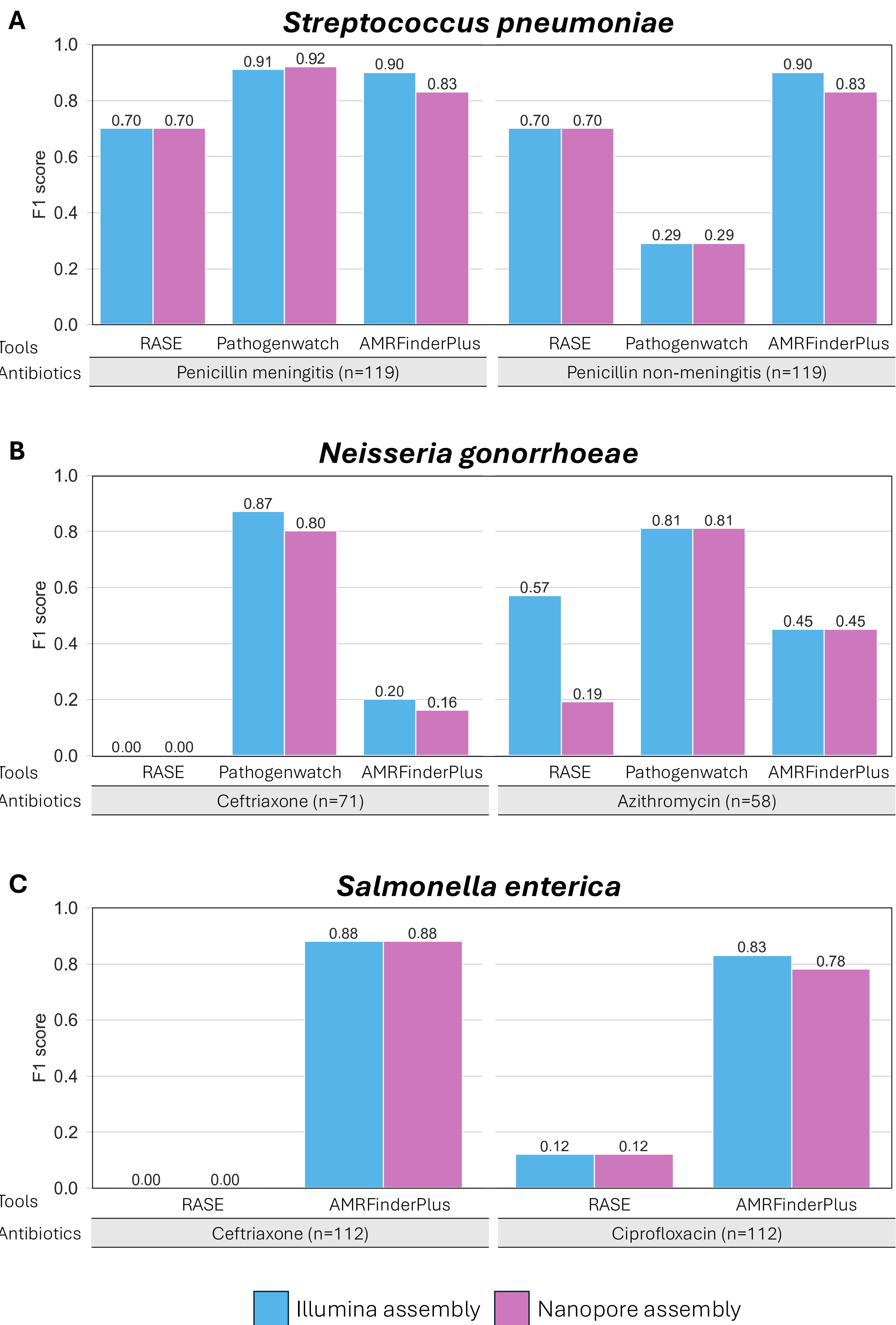


Fig.3: Predictive performance of AMR tools on (A) *Streptococcus pneumoniae*, (B) *Neisseria gonorrhoeae*, and (C) *Salmonella enterica* in-house datasets using Illumina assembly and Nanopore assembly for their first line of antibiotics.

F1 score = A metric that balances precision (Of all isolates predicted as resistant, how many are truly resistant) and recall (Of all truly resistant isolates, how many did the tool predict correctly).

Conclusion

NGS-based bioinformatics tools for AMR and serotyping demonstrated **rapid** and **comprehensive** pathogen characterization, offering advances in infectious disease surveillance and vaccine development.