Rapid phenotypic susceptibility testing from patient blood cultures with growth of Streptococci or other Gram-positive bacteria

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Background

Gram-positive bacteria are responsible for approximately half of all bacteraemia cases, with Streptococci accounting for a substantial, clinically relevant proportion of these cases. However, there are no rapid AST alternatives generating Minimum Inhibitory Concentration (MIC) data for Streptococci.

This study assessed the feasibility of testing clinical Gram-positive blood cultures (BCs) on the ASTar Instrument using development-stage consumables and software.

Conclusion

- Streptococcal and other Gram-positive patient blood cultures were tested with an overall categorical agreement > 98% compared to SoC.
- This is the first rapid AST direct from PBCs from Streptococci generating MIC values.

Materials and methods

Residual monomicrobial Gram-positive BCs from anonymised patients at Hvidovre Hospital, Denmark, were selected based on rapid MALDI-TOF results in combination with an algorithm* for sample selection (Fig 1).

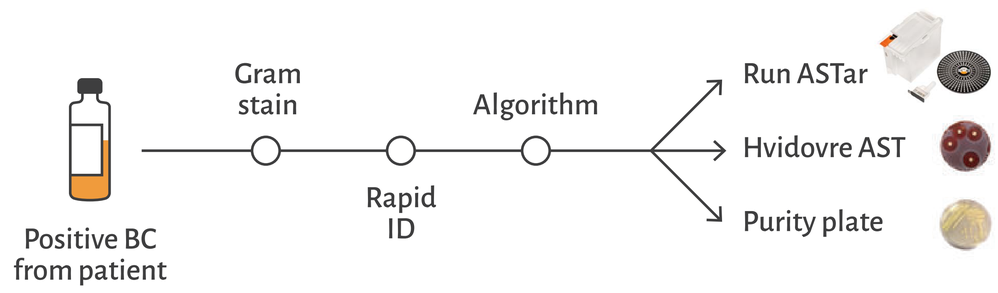

Fig 1. Clinical blood cultures, selected by rapid ID results and an algorithm for sample selection, were tested with ASTar in parallel to routine Hvidovre AST (overnight disc diffusion direct from BCs) and overnight purity plate culture.

After algorithm selection and removal of noneligible samples, complete ASTar results were obtained for 27 samples. One sample of *S. agalactiae* was included despite not meeting the inclusion criteria (tested > 16 h from positivity).

Standard of Care (SoC) was overnight disc diffusion direct from BCs. Results from ASTar and SoC were interpreted and compared using EUCAST breakpoints version 13.1, and discrepant results were assessed with third-party broth microdilution (BMD).

*Sample selection algorithm

To maximize the proportion of clinically relevant BCs tested, the following algorithm was applied for the three groups of typical contaminants:

1. Included Coagulase-negative Staphylococci were limited to:
   a. BC flasks with incubation times < 48 h
   b. ≥ 2 BC flasks in a set were positive
   c. For central venous or arterial line BCs, only BC flasks where central line/arterial BC flasks became positive > 2 h faster than simultaneously-drawn peripheral BCs were included. If no peripheral BC was taken, or if no peripheral BC flask was positive, the central line/arterial BC was excluded
2. Included non-hemolytic Streptococci were limited to:
   a. *Streptococcus anginosus* and *Streptococcus bovis*
   b. If endocarditis was suspected: All other nonhemolytic Streptococci were considered potential pathogens
3. Gram-positive rods
   a. Included: *Listeria monocytogens*, *Clostridum perfringens* and *Actinotignum schaalii*
   b. Excluded: Other species typically regarded as contaminants

Results

Both fastidious and non-fastidious species were represented among the included samples (Fig 2).

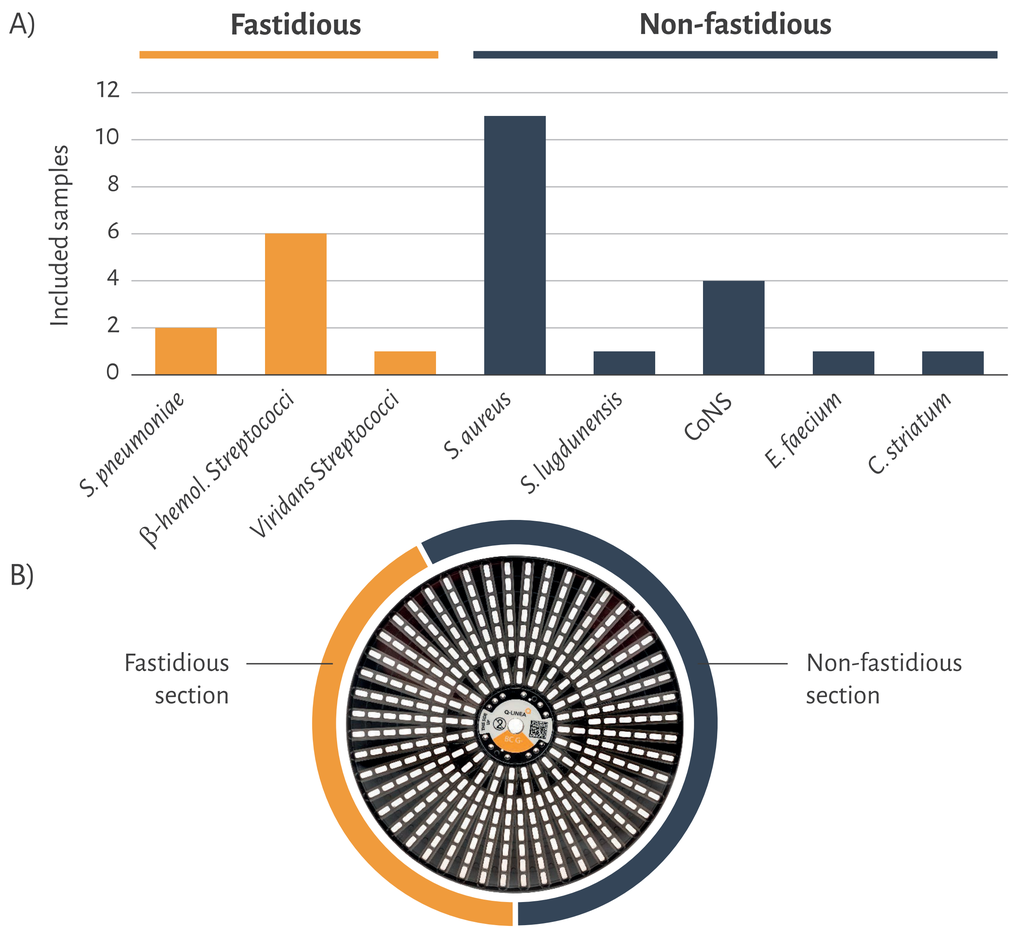

Fig 2. Both fastidious and non-fastidious bacteria were encountered among clinical samples. A) The species distribution of included samples covered both fastidious and non-fastidious bacteria. B) ASTar disc with sections for fastidious and non-fastidious antimicrobials, allowing start of AST without prior knowledge of species.

A total of 27 samples covering nine antimicrobials where results were available from both assays were compared (Table 1).

Table 1. Comparison of ASTar data and Hvidovre standard AST data after discrepancy resolution with BMD. NT: 'not tested'.

	Categorical Agreement (%)	
Antimicrobial	**Fastidious**	**Non-fastidious**
Ampicillin	2/2 (100%)	1/1 (100%)
Benzylpenicillin	9/9 (100%)	13/13 (100%)
Clindamycin	7/9 (78%)	16/17 (94%)
Erythromycin	8/8 (100%)	16/16 (100%)
Linezolid	8/8 (100%)	18/18 (100%)
Vancomycin	7/7 (100%)	17/17 (100%)
Rifampicin	NT	17/17 (100%)
Cefoxitin	NT	12/12 (100%)
Tetracycline	NT	1/1 (100%)
Total	**41/43 (95%)**	**111/112 (99%)**

Categorical agreement was 100% for all antimicrobial combinations except for Clindamycin with two Streptococci and one *Staphylococcus epidermidis*. For one of these datapoints, discrepancy resolution revealed that ASTar MIC was within essential agreement and on the breakpoint.

References

The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 13.1, 2023. https://www.eucast.org.

www.qlinea.com

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