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Evaluation of ASTar® remnant bacterial suspension for lateral flow immunoassay detection and differentiation of resistance mechanisms in Gram-negative resistant contrived blood cultures

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Background

Rapid detection of resistance mechanisms and phenotypic susceptibility results of Gram-negative (GN) positive blood cultures (PBC) can improve timely optimal antibiotic therapy and associated outcomes in patients with GN bloodstream infections (BSI).

The ASTar® System provides actionable phenotypic antimicrobial susceptibility testing (AST) results in approximately six hours (1) (Figure 1). During the run, a remnant bacterial suspension becomes available after approximately one hour. Extracting this remnant from the system consumables offers an opportunity for early adjunct testing, such as for lateral flow immunoassays (LFAs). (Research use only).

This proof-of-concept study evaluated whether the ASTar remnant bacterial suspension can be used as an input for LFAs to provide rapid detection of resistance mechanisms in several well-categorized Enterobacterales (ENT) and *Pseudomonas aeruginosa* (PsA) strains.

Figure 1. ASTar Instrument and kit consumables (cartridge and AST disc) *(not to scale)*.

Conclusions

This proof-of-concept study demonstrates that **remnant bacterial suspension from the ASTar System can be successfully used for rapid LFA-based detection of phenotypic gene expression resistance mechanisms,** supplementing the system's phenotypic AST results.

- High concordance was observed between LFA results and reference resistance genotypes for all Enterobacterales and *P. aeruginosa* strains tested.
- The presented procedure uses approximately 1/4 of available bacterial suspension, where the remainder of the remnant can be used in other applications.

An integrated workflow combining phenotypic AST and MIC results in ~6 hours of a positive blood culture with same-run resistance mechanism detection within 1-2 hours of instrument start may further improve early adequate antimicrobial therapy decision-making.

References

1. Göransson, J et al. "Performance of a System for Rapid Phenotypic Antimicrobial Susceptibility Testing of Gram Negative Bacteria Directly from Positive Blood Culture Bottles." Journal of clinical microbiology vol. 61,3 (2023): e0152522. doi:10.1128/jcm.01525-22
2. CDC & FDA Antimicrobial Resistance Isolate Bank: https://wwwn.cdc.gov/arisolatebank/
3. American Type Culture Collection (ATCC): https://www.atcc.org

Materials and methods

Workflow overview

The workflow takes less than ten minutes to prepare an LFA run from the ASTar cartridge sample remnant. (Figure 2).

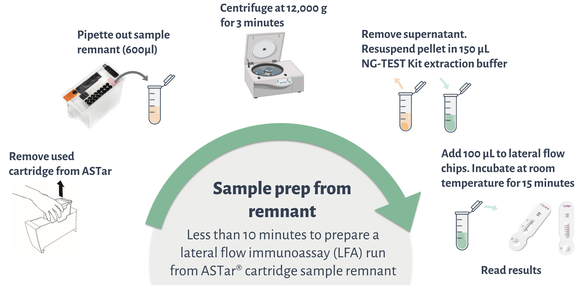

Figure 2. Sample preparation from ASTar cartridge remnant workflow.

Strains tested

Forty well-characterized isolates (Enterobacterales [ENT, n = 33] and *Pseudomonas aeruginosa* [PsA, n = 7]) from the CDC and FDA Antimicrobial Resistance Isolate Bank (AR Isolate Bank (2)) and the American Type Culture Collection (ATCC) Bank (3). See figure 3.

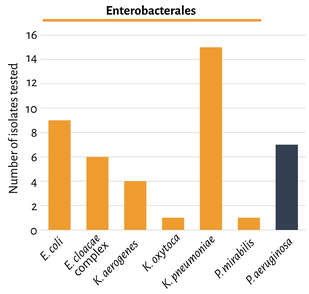

Figure 3. Pathogen distribution.

Workflow

Each isolate was processed and tested on the ASTar System (Q-linea). After one hour, the kit cartridge was retrieved. From this, the remnant bacterial suspension was collected and underwent genotypic testing using the NG-TEST CTX-M Multi, and/or NG-TEST CARBA-5.

Sample preparation

- 600 µL remnant bacterial suspension centrifuged at 12,000 g for 3 minutes
- Pellet re-suspended in 150 µL NG-TEST Kit extraction buffer
- 100 µL added to lateral flow chips. Incubated at room temperature for 15 minutes
- Results interpreted

For one isolate with an unexpected result, the NG-TEST Blood Culture Prep Kit (NG Biotech®) was used in addition to the full protocol.

Reference resistance mechanisms

Genotypic characterization as documented in CDC and FDA AR Isolate Bank.

Evaluated performance metrics

Preliminary Positive Predictive Value (PPV) and Negative Predictive Value (NPV).

Results

LFA testing results

Across 40 Gram-negative isolates, there was a mean remnant bacterial suspension volume of 2,825 µL (±140 µL [SD]), of which 600 µL was used for each LFA, sufficient for multiple LFAs.

Genotypic resistance profiles

ENT isolates (n = 33):
27/33 (82%) had a known carbapenemase genotype; 14/33 (42%) were CTX-M positive.

PsA isolates (n = 7):
6/7 (86%) had a known carbapenemase genotype.

ASTar x LFA Agreement

LFA results using ASTar remnant bacterial suspension showed 100% agreement with all reference CTX-M positive ENT (14/14, 100%) and all reference carbapenemase positive types except for identifying 3 of 4 (75%) reference OXA-48 positive isolates.

Performance (PPV and NPV)

CARBA-5

- **PPV:** 32/33 (97%)
- **NPV:** 4/4 (100%)

CTX-M Multi

- **PPV:** 14/14 (100%)
- **NPV:** 4/4 (100%)

Table 1. Comparison of LFA testing using ASTar remnant suspension (research use only) in genotypic-confirmed CDC AR Bank isolates.

Species and expected genotypes†	Reference (N)	ASTar sample prep (n)	% concordance (n/N)
Enterobacterales	**33**	**32**	**97%***
KPC	12	12	100%
NDM	4	4	100%
VIM	3	3	100%
IMP	4	4	100%
OXA-48	4	3*	75%
Negative CARBA-5	3	3	100%
CTX-M Group 1	11	11	100%
CTX-M Group 2	1	1	100%
CTX-M Group 9	2	2	100%
Negative CTX-M Multi test	3	3	100%
P. aeruginosa	**7**	**7**	**100%**
KPC	1	1	100%
VIM	2	2	100%
IMP	3	3	100%
Negative CARBA-5	1	1	100%

† A single isolate can have more than one genotype.
*One isolate that was OXA-48 positive by CDC AR Bank was OXA-48 negative from the same PBC by both ASTar prep and the NG- TEST Blood Culture PREP kit, but was OXA-48 positive when run from colony.

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