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SHORT COMMUNICATION

# Evaluation of an automated rapid phenotypic antimicrobial susceptibility testing (ASTar, Q-linea AB) applied directly on blood cultures bottles positive for Gram-negative pathogens

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SUMMARY

We evaluated the performance of a new rapid phenotypic antimicrobial susceptibility test (ASTar; Q-linea AB) on Gram-negative bacilli, directly from positive blood cultures bottles. MIC values obtained by the routine reference method (Microscan, Beckman Coulter) were compared to the ones provided by the tested method (ASTar). ASTar demonstrated an overall essential agreement of 98% and a category agreement of 96.1%. The overall rate of major errors and very major errors was 2.5% and 3.3%, respectively.
ASTar can represent a rapid, simple, and reliable method to speed up information about antimicrobial susceptibility of Gram-negative pathogens from positive blood culture bottles.

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Bloodstream infections (BSIs) represent a global burden of disease, with high morbidity and mortality rates (Fleischmann *et al*., 2016). Gram-negative bacilli (GNB) cause between one-quarter and one-third of BSIs worldwide (Santoro *et al*., 2020). In this context, early and optimal initiation of an active antibiotic treatment is one of the most critical issues to improve outcome of patients suffering from BSIs and to reduce healthcare-associated costs (Roncarati *et al*., 2021). Unfortunately, the global emergence of multi-drug resistance in GNB means that it is no longer possible to reliably predict antimicrobial susceptibility based on the identification of bacterial species alone (Perez *et al*., 2013). The spread of antimicrobial resistance may lead to the use of broad-spectrum therapies for empiric treatment of GNB BSIs, greatly increasing the risk of treatment inappropriateness, higher toxicity, higher costs, and further selection of bacterial resistance (Marquet *et al*., 2015). For this reason, antimicrobial susceptibility testing (AST), including MIC (minimum inhibitory concentration) determination using broth microdilution, is crucial for determining optimal antimicrobial treatment (Göransson *et al*., 2023). Over recent years, several methods have been developed to speed up the determination of MIC values and to detect antibiotic resistance in GNB from positive blood culture bottles (BCs) (Descours *et al*., 2018; Banerjee *et al*., 2021). In this study we evaluated the performance of a new rapid phenotypic AST (ASTar; Q-linea AB, Uppsala, Sweden) in GNB from positive BCs.

From September to October 2022, we analysed a total of 43 BCs collected from patients with Gram-negative bacteraemia at the Microbiology Unit of 'IRCCS Azienda Ospedaliero-Universitaria' of Bologna (Italy). Samples were randomly selected and only monomicrobial blood cultures were included. Samples were first processed with the standard routine workflow adopted by the laboratory (Foschi *et al*., 2016). Briefly, positive BC bottles (BACTEC, Becton Dickinson Diagnostic Systems, Franklin Lakes, NJ, USA) were analysed by Gram-staining, conventional subculture on solid media, and rapid identification using MALDI-TOF mass spectrometry (MS). When GNB were detected, the bacterial pellets obtained by BC broth centrifugation were inoculated onto chocolate agar. After incubation for 1.5 h at 35-37°C, bacterial growth was used for species identification by MAL-

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DI-TOF MS (Bruker Daltonics, Bremen, Germany) and for conventional antimicrobial susceptibility testing (AST) by microdilution assay (MicroScan Walkaway; Beckman Coulter, Atlanta, USA), requiring about 16 hours of incubation.

In parallel, the samples were analysed with ASTar system (Q-linea), following the manufacturer's instructions (Göransson *et al*., 2023). As described elsewhere (Göransson *et al*., 2023), ASTar is an automated rapid phenotypic AST method, performed directly from positive BCs (0.8-1 mL of sample from positive BC bottles is loaded into the cartridge), able to provide results of MIC values in about 6 hours. ASTar is based on time lapse microscopy to measure the concentration of bacteria present at given times in wells containing microdilutions in scaled broth of the antimicrobial agents.

ASTar uses concentration determination to produce a controlled final inoculum according to EUCAST guidelines, and microscopy is used to generate MIC values. Considering that ASTar cannot provide bacterial identification, species information needs to be entered into the system before results can be reported.

Retrospectively, we compared MIC values (interpreted following EUCAST guidelines, www.eucast.org) obtained by the routine reference method (Microscan) with those provided by the tested method (ASTar). Afterwards, the following parameters were evaluated:

1) 'Category Agreement' (CA), calculated as the total number of isolates tested using ASTar that yielded a MIC result in the same categorical interpretation (e.g., susceptible, resistant) of the reference method;

**Table** 1 - List of the strains included in the study. *For each group of microorganisms, the resistance rate obtained by the reference method (Microscan, Beckman Coulter) and stratified by the antimicrobials, is reported. In addition, the MIC values of resistant strains are provided. /=NOT TESTED or NOT REPORTED.*

| *Resistance rate* | *Escherichia coli (n=17)* | *Klebsiella spp. (n=10)* | *Enterobacter spp. (n=6)* | *Pseudomonas aeruginosa (n=5)* | *Serratia marcescens (n=3)* | *Acinetobacter baumannii (n=2)* |
|---|---|---|---|---|---|---|
| Amoxicillin-clavulanate | 64.7% (11/17) MIC: >32 (6); 32 (2); 16 (3) | 60% (6/10) MIC: >32 (5); 16 (1) | / | / | / | / |
| Ampicillin | 76.4% (13/17) MIC: >8 (13) | 100% (10/10) MIC: >8 (10) | / | / | / | / |
| Cefepime | 23.5% (4/17) MIC: >8 (4) | 60% (6/10) MIC: >8 (6) | 33.4% (2/6) MIC: >8 (2) | 0 | 0 | / |
| Cefotaxime | 29.4% (5/17) MIC: >32 (5) | 60% (6/10) MIC: >32 (6) | 50% (3/6) MIC: >32 (2); 16 (1) | / | / | / |
| Ceftazidime | 41.1% (7/17) MIC: >32 (2); 32 (2); 16 (1); 8 (2) | 40% (4/10) MIC: >32 (3); 8 (1) | 50% (3/6) MIC: >32 (1); 16 (1); 8 (1) | 0 | 0 | / |
| Ceftazidime-avibactam | 0 | 0 | 16.7% (1/6) MIC: >8 (1) | 0 | 0 | / |
| Ceftolozane-tazobactam | 0 | 30% (3/10) MIC: >64 (1); 64 (1); 4 (1) | 33.4% (2/6) MIC: >4 (2) | 0 | 0 | / |
| Ciprofloxacin | 23.5% (4/17) MIC: >1 (4) | 20% (2/10) MIC: >1 (2) | 33.4% (2/6) MIC: >1 (2) | 0 | 0 | 50% (1/2) MIC: >1 (1) |
| Ertapenem | 0 | 30% (3/10) MIC: >2 (3) | 50% (3/6) MIC: >2 (3) | / | 0 | / |
| Meropenem | 0 | 10% (1/10) MIC: >64 (1) | 16.7% (1/6) MIC: 16 (1) | 0 | 0 | 50% (1/2) MIC: >64 (1) |
| Amikacin | 0 | 10% (1/10) MIC: 16 (1) | 0 | 0 | 0 | 50% (1/2) MIC: >16 (1) |
| Gentamicin | 17.6% (3/17) MIC: >4 (3) | 20% (2/10) MIC: >4 (2) | 0 | / | 0 | 50% (1/2) MIC: >4 (1) |
| Piperacillin-tazobactam | 0 | 40% (4/10) MIC: >128 (3); 16 (1) | 33.3% (2/6) MIC: >16 (1); 16 (1) | 0 | 0 | / |
| Trimethoprim/ sulfamethoxazole | 41.1% (7/17) MIC: >4/76 (7) | 50% (5/10) MIC: >4/76 (5) | 16.7% (1/6) MIC: >4/76 (1) | / | 33.4% (1/3) MIC: >4/76 (1) | / |
| Tigecycline | 0 | / | 0 | / | / | / |

2) 'Essential Agreement' (EA), calculated by determining the number of test results that were within ±1 doubling dilution of the MIC value determined by the reference method;
3) 'major errors' (ME), indicating the percentage of susceptible isolates falsely determined by the tested method to be resistant, calculated with the number of susceptible isolates as the denominator, and
4) 'very major errors' (VME), indicating the percentage of resistant isolates falsely determined by the method tested to be sensitive, calculated with the number of resistant isolates as the denominator.

This study was conducted in compliance with the Helsinki Declaration and Italian regulations. No ethical approval was required since the study was purely observational, with data anonymized for analysis and presented in aggregated form.

Most of BCs analysed were positive for *Escherichia coli* (17/43) and *Klebsiella pneumoniae* (11/43), followed by *Pseudomonas aeruginosa* (5/43), *Serratia marcescens* (3/43), *Acinetobacter baumannii* (2/43), *Klebsiella aerogenes* (2/43), *Enterobacter spp.* (2/43), and *Klebsiella oxytoca* (1/43). The list of strains included in the analysis is shown in *Table 1*.

The ASTar system demonstrated an overall EA of 98% and a CA of 96.1% compared to the reference method. The overall rate of ME and VME was 2.5% and 3.3%, respectively. Among all the beta-lactams tested, piperacillin-tazobactam showed the lowest CA, whereas both carbapenems (i.e., ertapenem and meropenem) were characterized by an excellent CA (100%). Gentamicin and ceftolozane-tazobactam showed the highest rate of VME (16.7% and 20%, respectively). Detailed results are presented in *Table 2*.

Until now, only very few data are available about the ASTar system; thus, this work expands knowledge on the accuracy and performance of this new method.

Our data are in line with the recent results presented by Göransson *et al.* showing that the ASTar system delivers reproducible results with overall EA and CA of >95%, with an overall rate of major discrepancies of 0.9%, and that of very major discrepancies of 2.4% (Göransson *et al.*, 2023). In agreement with our observations, the same authors found that gentamicin showed the highest rate of VME (>16%) (Göransson *et al.*, 2023).

ASTar can represent a rapid, simple, and reliable method to speed up information about antimicrobial susceptibility of GNB from positive BC bottles. These included the Gram-negative pathogens responsible for most cases of bacteraemia/sepsis, such as *Escherichia coli* and *Klebsiella pneumoniae* (Ohnuma *et al.*, 2023). Further studies, including a larger panel of samples and species, are needed for thorough evaluation of the cost-benefit ratio of this method applied to the management of septic patients. Even though other works will be necessary to assess the clinical outcomes, laboratory workflow, or health economic benefits of the ASTar system, the rapid availability of MIC

**Table 2** - Accuracy study results for each antimicrobial. *CA=Category Agreement'; EA= 'Essential Agreement'; ME= major errors: percentage of susceptible isolates falsely determined by the tested method to be resistant, calculated with the number of susceptible isolates as the denominator; VME= 'very major errors: percentage of resistant isolates falsely determined by the method tested to be sensitive, calculated with the number of resistant isolates as the denominator. Only valid microorganisms-drug combinations were included in the analysis.*

| *Antimicrobial agent* | *EA* | *CA* | *ME* | *VME* |
|---|---|---|---|---|
| Ampicillin | 100% (17/17) | 100% (17/17) | 0% | 0% |
| Amoxicillin-clavulanate | 96.3% (26/27) | 88.9% (24/27) | 22.2% (2/9) | 5.9% (1/17) |
| Piperacillin-tazobactam | 97.6% (40/41) | 90.2% (37/41) | 8.6% (3/35) | 0% |
| Cefepime | 100% (34/34) | 97.4% (37/38) | 0% | 0% |
| Cefotaxime | 93.9% (31/33) | 93.9% (31/33) | 0% | 7.1% (1/14) |
| Ceftazidime | 97.6% (40/41) | 92.7% (38/41) | 0% | 0% |
| Ceftazidime-avibactam | 100% (41/41) | 97.6% (40/41) | 0% | 0% |
| Ceftolozane-tazobactam | 97.5% (39/40) | 95.0% (38/40) | 2.9% (1/35) | 20% (1/5) |
| Ciprofloxacin | 97.6% (42/43) | 90.7% (39/43) | 0% | 0% |
| Amikacin | 100% (43/43) | 100% (43/43) | 0% | 0% |
| Gentamicin | 97.4% (37/38) | 97.4% (37/38) | 0% | 16.7% (1/6) |
| Tigecycline | 100% (36/36) | 100% (36/36) | 0% | 0% |
| Trimethoprim/sulfamethoxazole | 97.2% (35/36) | 97.2% (35/36) | 4.5% (1/22) | 0% |
| Ertapenem | 97.2% (35/36) | 100% (36/36) | 0% | 0% |
| Meropenem | 97.7% (42/43) | 100% (43/43) | 0% | 0% |
| Total | 98.0% | 96.1% | 2.5% | 3.3% |

values/AST results could probably reduce the length and cost of hospitalization, as well as mortality rates.

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