# **Gram-negative blood stream infections: prospects and challenges of rapid antimicrobial susceptibility testing**

1. **Introduction**

Over the last decade, Gram-negative bacteria have become the most predominant pathogens in blood stream infections (BSIs), accounting for over 1.8 million cases in Europe and North America [1]. At the same time, effective antimicrobial treatment options are increasingly limited due to the burden of antimicrobial resistance. Therefore, providing early appropriate antibiotic has been suggested as key to improve outcomes in patients with Gram-negative BSIs, in order to reduce inappropriate antibiotic usage that further contributes to antimicrobial resistance. New automated systems use novel technologies to provide antimicrobial susceptibility results in times as short as 30 minutes to 8 hours after blood culture positivity. This could allow clinicians to start appropriate antimicrobial treatment much earlier than is possible now.

1. **Body**

Rapid antimicrobial susceptibility testing (AST) for the diagnosis of Gram-negative BSIs can be broadly divided into two categories. These are either the direct detection of resistance genes, known as genotypic or molecular testing or the *in-vitro* determination of antimicrobial susceptibility by demonstrating the suppression of growth of bacteria by antibiotics, known as phenotypic testing. A number of genotypic or molecular technologies have been developed to provide antimicrobial resistance information directly from clinical samples in Gram-negative BSIs [2] (**Table 1**), such as i) nucleic acid amplification tests (NAATs), designed to detect different gene targets identified as responsible antimicrobial resistance, such as by polymerase chain reaction (PCR) [3] [4] [5]. ii) DNA-microarray-based hybridization, in which single-stranded DNA probes are designed to bind to the target antimicrobial resistance genes during a hybridization process [6-8].

iii) loop-mediated isothermal amplification (LAMP) [9]. iv) metagenomic whole-genome-sequencing which can detect all antimicrobial resistant genes present in the bacterial genome and provide information about virulence. However, the proof-of-concept system for this molecular-genetic approach has not been yet commercialized for clinical use.

To date, commercially available genotypic testing platforms are able to provide rapid results ranging from 30 minutes to 2 hours after blood culture positivity, directly from blood culture bottles or from a whole blood sample. However, important drawbacks include that the detection is limited to a particular set of antimicrobial resistance encoding genes, as only a few resistance genes have been associated with phenotypic resistance. This means many resistant bacteria would not be identified as resistant by only detecting a limited number of resistance genes. Consequently, due to the great diversity of resistance mechanisms in Gram-negative BSI, genotypic testing may be helpful for escalating antimicrobial therapy if resistance is detected, but it may not provide sufficient information to “rule out” resistance to give assurance to switch to using a narrow spectrum antibiotic or to provide complete susceptibility information [10]

Rapid phenotypic AST methods offers two main advantages compared to genotypic testing methods. First, both antimicrobial resistance and susceptibility are predicted and second, the degree of susceptibility of a bacterial isolate to antimicrobial agents can be quantified. Nevertheless, time to results may be one of the main limitations for phenotypic methods, since antimicrobials need to be present for enough time to inhibit the growth of bacteria. This may take between 3 and 8 hours depending on several factors such as the antimicrobial mechanism of action, concentration and stability or bacterial inoculum among others. Phenotypic rapid susceptibility techniques include i) Fluorescence in situ hybridization (FISH) which detects nucleic acid sequences combined with real-time morpho kinetic cellular analysis by using dark-field microscopy to provide susceptibility results [11]; ii) light-scattering technology is able to record turbidity of growing bacteria in special liquid media [12]; iii) microscopic imaging analysis with microfluidic chip technology [13]; iv) detection of microbial cell changes by flow cytometric analysis [14]; v) culture-based approach using a rapid antimicrobial disc diffusion test directly performed from blood culture bottles [15]. **Table 1.**

Several limitations common to all current new rapid susceptibility technologies include the inability to accurately analyse polymicrobial samples. In addition, because of the novelty and lack of familiarity with new rapid susceptibility tests, many front-line clinicians may not fully rely on AST results provided by the new systems and subsequently delay antimicrobial treatment decisions until conventional susceptibility results are available several days later. In this regard, simply introducing a rapid testing technology will not, by itself, improve patient outcomes and antimicrobial stewardship programs would be needed to facilitate the implementation of new rapid susceptibility testing and provide decision support for clinicians treating patients with Gram-negative blood stream infections [16].

Furthermore, a recent systematic review of randomized controlled trials comparing rapid and standard antimicrobial susceptibility testing for guiding antibiotic treatment in patients with BSIs, showed no improvement in clinical outcomes in terms of 30-day mortality, length of stay or time to appropriate antibiotic. However, this review suggested that rapid phenotypic testing may shorten the time to appropriate antibiotic [17].

In conclusion,an increasing number of rapid AST technologies are continuously emerging to meet the need of improving the diagnosis and management of patients with Gram-negative BSIs. However, the current available platforms are still far from giving the quick and accurate answer clinicians need to choose the right antimicrobial when a patient presents with sepsis or BSI. Further research should explore pitfalls and hurdles of rapid AST platforms in real clinical settings and evaluate its clinical performance. Finally, joint efforts among clinicians, microbiologists and antimicrobial stewardship teams will be required to start seeing all desirable positive outcomes in patients when using these rapid technologies.

**Declaration of interest**

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

**Author’s contributions**

All authors have substantially contributed to the conception, design and writing of the review article

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**Table 1.** Rapid genotypic and phenotypic susceptibility commercial platforms for the diagnosis of Gram-negative BSIs from positive blood cultures or from whole blood samples. Specifications of the assays were collected from the literature. Abbreviations: GP: Gram-positive; GN: Gram-negative; ESBL: Extended spectrum beta-lactamase.Abbreviations: GP: Gram-positive; GN: Gram-negative; CFT: cefotaxime; ERY: erythromycin; AMP: ampicillin; AMK: amikacin; ATM: aztreonam; CAZ: ceftazidime, CIP: ciprofloxacin; CRO: ceftriaxone; ERT: ertapenem; FEP: cefepime; GE: gentamycin; MEM: meropenem; SAM: ampicillin/sulbactam; TOB: tobramycin; TZP: piperacillin/Tazobactam; AMC: amoxicillin/clavulanate; CST: colistin; IPM: imipenem; TMP-SMZ: trimethoprim-sulfamethoxazole; MIN: minocycline; CTZ-AVI: ceftazidime/avibactam; C\_T: ceftolozane/tazobactam. BC: blood culture.

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| Rapid genotypic susceptibility testing | | | | | | | | |
| System | **Technology** | **Organism** | **Resistance genes** | **Assay time/ sample type** | **Sensitivity** | **Regulatory status** | | **Cost per test/ equipment** |
| [Biofire BCID2](https://pubmed.ncbi.nlm.nih.gov/32305272/)  [(Biofire)](https://pubmed.ncbi.nlm.nih.gov/32305272/) [3] | Nucleic-acid amplification-based method (multiplex PCR) | 14 GN | ESBL:CTX-M Carbapenemases: IMP , KPC , OXA−48−like , NDM , VIMColistin resistance*: mcr-1* | 1 h  BC bottle | 94% | FDA-cleared and CE-marked | |  |
| ~~[LightMix](https://pubmed.ncbi.nlm.nih.gov/27566313/)~~  ~~[(Roche diagnostics)](https://pubmed.ncbi.nlm.nih.gov/27566313/)~~ | ~~Nucleic-acid amplification-based method~~ | ~~No~~ | ~~Carbapenemases: KPC, NDM, VIM, IMP, OXA 48~~ | ~~2 h~~  ~~BC bottle~~ | ~~99%~~ | ~~FDA-cleared~~ | |  |
| [Verigene](https://pubmed.ncbi.nlm.nih.gov/25994165/)  [(Luminex)](https://pubmed.ncbi.nlm.nih.gov/25994165/) [6] | DNA-Microarray based-method | 9 GN | ESBL: CTX-M Carbapenemases: KPC, NDM, VIM,  IMP, OXA-48, OXA-23, OXA-24/40, OXA-58 | 2.5 h  BC bottle | 89–100% | CE-marked | | €€ / €€€ |
| [T2Bacteria](https://pubmed.ncbi.nlm.nih.gov/31083728/)  [(Biomedica)](https://pubmed.ncbi.nlm.nih.gov/31083728/) [7] | Combined (T2 Magnetic resonance-based method after DNA hybridization) | 4 GN | Carbapenemases: KPC, CTX-M-14, OXA-48, CTX-M-15, NDM, AmpC (CMY), VIM, AmpC (DHA), IMP | 4.1 h  Whole blood | 91% | FDA-cleared and CE-marked | | €€€ / €€€€€€€ |
| [ePlex](https://pubmed.ncbi.nlm.nih.gov/30487304/)  [(GenMark)](https://pubmed.ncbi.nlm.nih.gov/30487304/) [8] | Combined method (multiplex PCR and DNA-microarrays) | 21 GN | ESBL : CTX-m. Carbapenemases : OXA-48, OXA-23, KPC,  NDM, VIM, IMP | 30 min  BC bottle | 96% | CE-marked | | €€€€ / €€€ |
| [Sepsis Flow chip](https://pubmed.ncbi.nlm.nih.gov/28542614/)  [(Master Diagnostic)](https://pubmed.ncbi.nlm.nih.gov/28542614/) [18] | Combined method (multiplex PCR and hybridation or DNA-microarrays) | 10 GN | ESBL: CTX-M, SHV. Carbapenemases: KPC,  NDM,  IMP,  OXA-48, OXA-23, OXA 24/40,  OXA-51,  OXA-58 | 3 h  BC bottle | 93% | CE-marked | |  |
| Eazyplex SuperBug CRE  (AmplexDiagnostics) [9] | Loop-mediated isothermal amplification (LAMP) |  | ESBL: CTX-M, SHV, TEM. AmpC. Carbapenemases: KPC, NDM, OXA-48, VIM. | 20 min  BC bottle | 88% | CE-marked | | €€€ / €€€ |
| Unyvero BCU (Curetis) [4] | Multiplex PCR |  | ESBL: CTX-M  Carbapenemases: KPC, IMP, NDM, OXA-48, OXA-23, OXA-58, OXA 24-40, VIM | 5 h  BC bottle | 97% | CE-marked | |  |
| AID carbapenemase (AIDGmbH) [5] | Multiplex PCR |  | Carbapenemases: KPC, NDM, VIM, IMP, OXA-48-like | 3 hours  BC bottle | 100% | CE-marked | |  |
| Rapid phenotypic susceptibility testing | | | | | | | | |
| System | **Technology** | **Organism** | **Antimicrobials available** | **Assay time/ sample type** | **CA** | | **Regulatory status** | **Cost per test/ equipment** |
| [Pheno Test BC](https://pubmed.ncbi.nlm.nih.gov/29474660/)  [(Accelerate diagnostic)](https://pubmed.ncbi.nlm.nih.gov/29474660/) [11] | Time-lapse imaging of bacterial cells on dark-field microscopy. Antimicrobial susceptibility based on Morphokinetic cellular analysis. | 8 GN | Multiple antibiotics: AMK, ATM, CAZ, CIP, CRO, ERT, FEP, GE, MEM, SAM, TOB, TZP, TMP-SMZ CTZ-AVI, C\_T | 7 h  BC bottle | 95% | | FDA-cleared and CE-marked | €€€ |
| [Alfred AST](https://pubmed.ncbi.nlm.nih.gov/31783787/)  [(AliFAX)](https://pubmed.ncbi.nlm.nih.gov/31783787/) [12] | Light scattering | No | Multiple antibiotics: AMK, ATM, CAZ, CIP, CRO, ERT, FEP, GE, MEM, TZP, AMC | 3-5 h  BC bottle | 96% | | CE-marked | €€ |
| [QMAC-dRAST](https://pubmed.ncbi.nlm.nih.gov/32205178/)  [(QuantaMatrix)](https://pubmed.ncbi.nlm.nih.gov/32205178/) [13] | Microscopic imaging with microfluidic technology | No | Multiple antibiotics: AMK, ATM, CAZ, CIP, CRO, ERT, FEP, GE, MEM, SAM, TOB, TZP, CST, IPM, TMP-SMZ, MIN, CTZ-AVI, C\_T | 3-6 h  BC bottle | 93% | | CE-marked |  |
| [Fastinov](https://pubmed.ncbi.nlm.nih.gov/29312169/)  [(Portugal)](https://pubmed.ncbi.nlm.nih.gov/29312169/) [14] | Detection of microbial cell changes by flow cytometric analysis |  | Multiple antibiotics: AMK, AMC, CFT, CAZ, CIP, GE, CST, TZP, MEM | 80 min  BC bottle | 98% | | CE-marked |  |
| [EUCAST Rapid disc diffusion](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7069491/) [15] | Manual disc diffusion | No | Multiple antibiotics: AMK, GE, TOB, CFT, CAZ, CIP, TZP, MEM | 4- 8h  BC bottle | 96% | | Not CE/ FDA |  |