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Rapid determination of antimicrobial susceptibility of Gram-negative bacteria from clinical blood cultures using a scattered light-integrated collection device

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Abstract

Background. A bloodstream infection (BSI) presents a complex and serious health problem, a problem that is being exacerbated by increasing antimicrobial resistance (AMR).

Gap Statement. The current turnaround times (TATs) for most antimicrobial susceptibility testing (AST) methods offer results retrospective of treatment decisions, and this limits the impact AST can have on antibiotic prescribing and patient care. Progress must be made towards rapid BSI diagnosis and AST to improve antimicrobial stewardship and reduce preventable deaths from BSIs. To support the successful implementation of rapid AST (rAST) in hospital settings, a rAST method that is affordable, is sustainable and offers comprehensive AMR detection is needed.

Aim. To evaluate a scattered light-integrated collection (SLIC) device against standard of care (SOC) to determine whether SLIC could accelerate the current TATs with actionable, accurate rAST results for Gram-negative BSIs.

Methods. Positive blood cultures from a tertiary referral hospital were studied prospectively. Flagged positive Gram-negative blood cultures were confirmed by Gram staining and analysed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, Vitek 2, disc diffusion (ceftriaxone susceptibility only) and an SLIC device. Susceptibility to a panel of five antibiotics, as defined by European Committee on Antimicrobial Susceptibility Testing breakpoints, was examined using SLIC.

Results. A total of 505 bacterial–antimicrobial combinations were analysed. A categorical agreement of 95.5% (482/505) was achieved between SLIC and SOC. The 23 discrepancies that occurred were further investigated by the broth microdilution method, with 10 AST results in agreement with SLIC and 13 in agreement with SOC. The mean time for AST was 10.53±0.46 h and 1.94±0.02 h for Vitek 2 and SLIC, respectively. SLIC saved 23.96±1.47 h from positive blood culture to AST result.

Conclusion. SLIC has the capacity to provide accurate AST 1 day earlier from flagged positive blood cultures than SOC. This significant time saving could accelerate time to optimal antimicrobial therapy, improving antimicrobial stewardship and management of BSIs.

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Keywords: antimicrobial resistance; antimicrobial stewardship; antimicrobial susceptibility testing; blood culture; bloodstream infection; rapid diagnostics.

Abbreviations: ABC, antimicrobial-bacterial combination; AMC, amoxicillin-clavulanic acid; AMR, antimicrobial resistance; AST, antimicrobial susceptibility testing; AUC, area under the curve; BHI, brain heart infusion; BMD, broth microdilution; BSI, bloodstream infection; CA, categorical agreement; CIP, ciprofloxacin; CRO, ceftriaxone; dB, decibels; ESKAPE, enterococcus faecium, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Enterobacter* species; EUCAST, European Committee on Antimicrobial Susceptibility Testing; GEN, gentamicin; GN, gram-negative; GNB, gram-negative bacteria; GNBC, gram-negative blood culture; Maj, major error; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MDR, multidrug resistance; MEM, meropenem; MIC, minimum inhibitory concentration; NHS, National Health Service; PBS, phosphate buffered saline; rAST, rapid antimicrobial susceptibility testing; SLIC, scattered light-integrated collection; SOC, standard of care; TAT, turnaround time; TSB, tryptic soy broth; Vmj, very major error.

One supplementary figure and two supplementary tables are available with the online version of this article.

Impact Statement

Phenotypic rapid antimicrobial susceptibility testing (rAST) offers an opportunity to accelerate time to detect antimicrobial-resistant infections and to support evidence-based treatment decisions. This is the first study to examine the performance of a scattered light-integrated collection (SLIC) device against conventional antimicrobial susceptibility testing (AST) methods of either disc diffusion, Vitek 2 or broth microdilution in a clinical setting. Bloodstream infections (BSIs) caused by antimicrobial-resistant Gram-negative bacteria are increasingly common and heighten the risk of treatment failure, health complications and death. In this study, bacterial resistance to at least one antibiotic class was detected in 59.4% of blood cultures. Promoting antibiotic stewardship and improving survival in BSI patients necessitate rapid detection of antimicrobial resistance to ensure that targeted antimicrobial treatment is commenced on day 1 of admission. This prospective comparison demonstrated that this is achievable using SLIC and served to reduce the current turnaround times by 44.5%. The high comparability of AST agreement between SLIC and conventional methods (95.5%) supports this technology to provide accurate rAST within 2 h of a blood culture flagging positive. The study also demonstrated that SLIC could integrate into the current diagnostic workflow to streamline antibiotic prescribing in real time. The affordability, ease of use and minimal hands-on time of this technology are key attributes missing from many technologies on the market today.

DATA SUMMARY

The authors confirm all supporting data, code and protocols have been provided within the article or through supplementary data files.

INTRODUCTION

Antibiotics, the 'magic bullets' for the treatment of infection, have saved and continue to save countless lives each year. Without their efficacy, modern medicine faces a very grim future, with antimicrobial resistance (AMR) projected to cause 10 million deaths by 2050 [1].

One of the main driving forces of AMR is the lack of rapid diagnostics to inform evidence-based treatment decisions [2]. Empirical antimicrobial therapy is the reality of practice but is only effective if the bacterial infection is susceptible to the antibiotic prescribed. As the effectiveness of first-line antimicrobial therapy continues to fall with the rise of AMR, it is becoming harder to predict antibiotic susceptibility patterns and achieve appropriate empirical coverage [3].

A bloodstream infection (BSI) is a systemic infection associated with high morbidity and mortality [4]. The timeliness of appropriate antimicrobial therapy is of the utmost importance for patient survival, but up to 40–70% of patients with BSIs receive inappropriate or inadequate antimicrobial therapy [5]. Blood cultures are considered to be the 'gold standard' diagnostic tool for the detection of BSI. Following the detection of BSI, a secondary overnight culture is required for most antimicrobial susceptibility testing (AST) methods. This is normally performed using conventional culture methods or automated AST platforms such as Vitek 2.

The current BSI diagnostic workflow adopted by the UK National Health Service (NHS) is expensive, labour-intensive and time-consuming, with a target turnaround time (TAT) of 5 days. Similarly, an average TAT of 6 days for AST reporting has been observed in US hospitals [6, 7]. This timeline does not match the need to administer optimal treatment quickly. Delayed optimal therapy prolongs exposure to infection and is associated with irreversible tissue damage. The likelihood of survival is directly proportional to the timeliness of appropriate therapy [6, 8, 9]. Thus, earlier informed treatment decisions are vital to improving the outlook for patients with BSIs. This requires the development and implementation of rapid AST (rAST) [10–12].

The scattered light-integrated collection (SLIC) device has been designed to offer an inexpensive, simple and highly sensitive rAST detection system. This patented technology works by monitoring minute changes in bacterial growth by analysing total light scatter in real time and allows differentiation between susceptible and resistant bacterial populations within minutes (patents GB201502194D0, GB201619509D0 and EP3759464A1). A detailed overview of the technology has been published previously [13].

For this proof-of-concept study, we focus on BSIs caused by Gram-negative bacteria (GNB) due to their high mortality and morbidity, growing incidence, and the risk of multidrug resistance and treatment failure [3, 4, 14]. Another reason is the isolation of GNB from blood culture almost always represents true bacteraemia. Blood culture contamination from skin flora or indwelling venous catheters is a common challenge for interpreting the clinical significance of many Gram-positive isolates. Furthermore, in these cases, the time to positivity is much longer, and this dilutes the importance of rapid diagnosis.

The aim of this research was to compare the use of SLIC against standard of care (SOC) in a busy hospital setting to determine whether SLIC could expedite the current TAT with actionable, accurate rAST results for Gram-negative BSIs.

METHODS

Study design

A prospective cohort study was conducted at Ninewells Hospital and Medical School, Dundee, Scotland, UK, an 862-bed teaching hospital. From 17 January 2019 to 9 May 2019, blood cultures on the BacT/ALERT system (bioMérieux, Basingstoke, UK) that signalled positive and were confirmed to contain GNB by Gram stain were included in the study. Our aim was to process approximately 100 Gram-negative blood cultures.

BacT/ALERT system

Patient samples were collected in either BacT/ALERT FA plus media (30 ml), BacT/ALERT FN plus media (40 ml) or BacT/ALERT PF plus media (30 ml). The date and time the sample was taken were recorded. Blood cultures were collected in pairs, except for paediatric samples, where only one blood culture is required for coverage of both aerobic and anaerobic organisms. Each blood culture bottle was automatically read every 10 min by the BacT/ALERT system according to the manufacturer's instructions. The time to flag positive was recorded for all samples.

rAST from positive blood culture using SLIC

The SLIC device is an innovative combination of laser light scattering, locked signal and an integrating detection space. The shape of the integrating space allows light scatter in all directions to be analysed, offering higher resolution compared to conventional forward or side scatter. Monitoring the total light scatter allows the heightened detection of small changes in the bacterial population, including bacterial replication, growth inhibition and cell death. Any changes in the bacterial population are reflected in the total light scatter output and this is converted into decibels (dB) by the photodetectors. This allows the unique scattering patterns to be quantified across six wells simultaneously (patents GB201502194D0, GB201619509D0 and EP3759464A1).

The SLIC v7.0 method was run in parallel with the SOC workflow for blood culture (Fig. 1) [13]. All positive blood cultures were Gram stained after flagging positive. Following confirmation that the blood culture was positive for GNB, a volume of 1 ml was aliquoted from each blood culture for the SLIC analysis. Blood cultures were diluted 1:10 into sterile phosphate-buffered saline (PBS) and then 1:50 into SLIC in pre-warmed tryptic soy broth (TSB). Each SLIC run included a positive control containing 20 µl patient sample, 100 µl PBS and 880 µl TSB broth, and the remaining five wells contained 20 µl patient sample, 100 µl of one

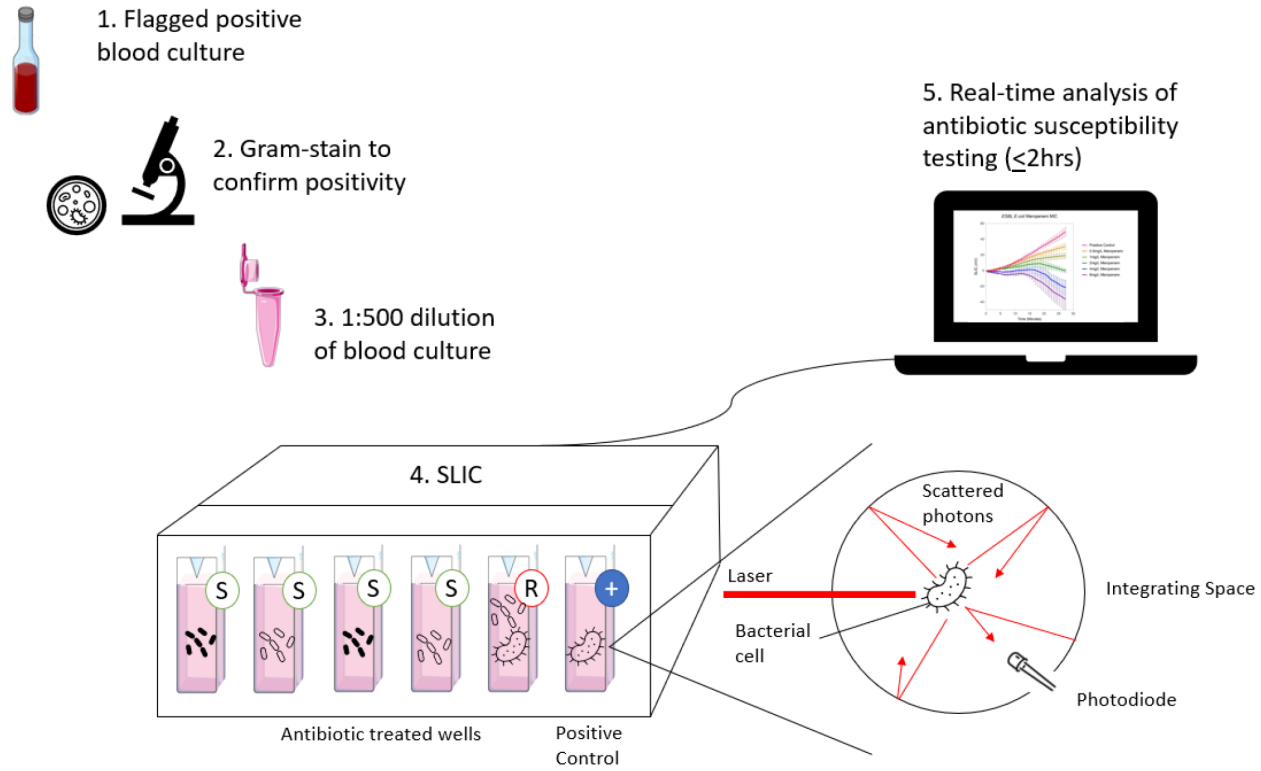

Fig. 1. The SLIC rAST workflow consists of 10 min hands-on time and five simple steps and can be completed within 2 h. In this trial, each AST run consisted of a positive control and five antibiotic-treated wells to analyse bacterial susceptibility directly from positive blood culture in real time.

of the five selected antibiotics and 880 µl TSB broth. The AST panel was selected by two consultant clinical microbiologists to detect for bacterial resistance against five different modes of antimicrobial action and is not necessarily reflective of the choice of therapy. The antibiotic panel and susceptibility breakpoints were as follows: 1 mg l^{-1} ceftriaxone (CRO) (Sigma Aldrich, Dorset, UK), 2 mg l^{-1} meropenem (MEM) (Sigma Aldrich), 0.5 mg l^{-1} ciprofloxacin (CIP) (Sigma Aldrich), 4 mg l^{-1} gentamicin (GEN) (Alfa Aesar, Heysham, UK) and 8 mg l^{-1} amoxicillin–clavulanic acid (AMC) (Sigma Aldrich). All antibiotics were stored at 5°C. Each antibiotic was dissolved according to the manufacturer's instructions. rAST was performed on SLIC for 120 min. The analysis time and AST outcome for both Vitek 2 (bioMérieux) and SLIC were recorded. AST results were interpreted in line with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints 2019 guidelines (v 9.0) [15].

Discrepancy analysis

Broth microdilution (BMD) was used to further investigate AST discrepancies between SLIC and SOC. Each sample with a discrepant result was inoculated from glycerol stock onto brain heart infusion (BHI) agar incubated overnight at 37°C in aerobic conditions. The bacterial concentration was diluted to 10^6 c.f.u. ml^{-1} in TSB confirmed by spectrophotometry and c.f.u. ml^{-1} dilution plate. A volume of 160 µl of TSB, 20 µl of bacterial culture and either 20 µl of antibiotic or 20 µl of PBS (positive control) was pipetted into each well of the microwell plate to achieve a final bacterial concentration of 10^5 c.f.u. ml^{-1}. MIC was determined as the lowest concentration of antibiotic that completely inhibited growth. EUCAST breakpoints (2019, v 9.0) were used [15].

Data collection and analysis

Workflow

The sample journey from the patient to the AST outcome was analysed based on AST agreement and TAT for both SOC and SLIC. The sample transfer time from patient to BacT/ALERT, the time to blood culture positivity on BacT/ALERT and the time elapsed between a blood culture flagging positive to being processed on Vitek 2 were collected to determine the overall TAT from patient sampling to AST outcome (Fig. 2). AST analysis time on Vitek 2 and SLIC was directly compared for each sample to calculate the time difference to AST result.

Antimicrobial susceptibility testing

AST categorical agreement of SLIC was analysed retrospectively against Vitek 2. The Vitek 2 AST-N381 card was selected for all Enterobacterales and AST-N383 card for non-fermenters. CRO susceptibility was determined by disc diffusion as per local guidelines. Isolates that fell under 'I – susceptible, increased exposure' were not included in the comparison. AST categorical agreement was defined as either:

(1) 'Agreed' – SOC and SLIC shared the same AST result.
(2) 'Major error (Maj)' – SOC classified the isolate as susceptible when SLIC called the isolate resistant.
(3) 'Very major error (Vmj)' – SOC classified the isolate as resistant when SLIC called the isolate susceptible.

The rate of Vmj errors was calculated by dividing the number of Vmj errors by the number of resistant isolates multiplied by 100. The rate of Maj errors was calculated by dividing the number of Maj errors by the number of susceptible isolates multiplied by 100.

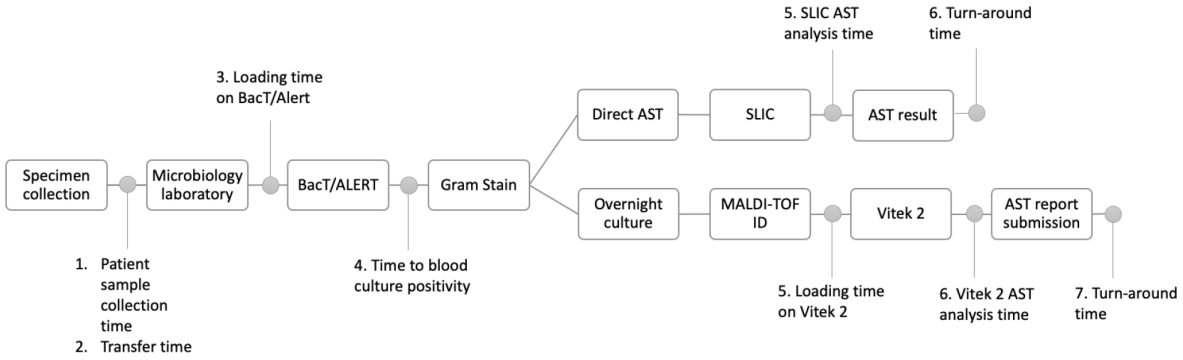

Fig. 2. Time to event definition. Data were collected at each of the following stages for SOC and the SLIC workflow: (1) the patient sample collection time; (2) time of laboratory sample receipt (transfer time); (3) time of loading sample on BacT/ALERT system and (4) time to blood culture positivity. Additional data collected for SOC included (5) time of loading sample on Vitek 2; (6) Vitek 2 AST analysis time and (7) AST result/final AST report submission (TAT). Additional data for the SLIC workflow included (5) SLIC AST analysis time and (6) AST result output (TAT).

AMR

The incidence of AMR for each antibiotic class, bacterial species and occurrence of multidrug resistance (MDR) was recorded in the study.

Statistical analysis

Continuous data points are expressed as mean±standard error of mean (SEM) or medians and interquartile ranges. The SLIC AST result was determined by calculating the area under the curve (AUC) of the positive control and antibiotic-treated cells. Independent *t*-tests at 95% confidence levels were used to determine statistical significance between AUC means. Bacteria were classified as 'susceptible' when the mean AUC was statistically significantly reduced in the presence of an antibiotic in comparison to the mean AUC of the positive control. Bacteria were classified as 'resistant' when the AUC means with and without antibiotic treatment were not statistically significantly different.

Categorical data are reported as frequencies and percentages. McNemar's test was used to assess whether a statistically significant difference was present between the AST outcome of SLIC and SOC. $P<0.05$ was considered significant. All data analysis was performed using GraphPad Prism v8.0.2.

RESULTS

Detection of Gram-negative BSI

A total of 4478 blood cultures were processed from 17 January to 7 May 2019. During this time, 631 blood cultures flagged positive on the BacT/ALERT system. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and Vitek 2 identified 37.6% of positive blood cultures to contain GNB and 62.4% to contain Gram-positive bacteria. The most prevalent Gram-negative bacterium was *Escherichia coli*, accounting for 59.9% of bacteraemia episodes. A total of 101 positive blood cultures met the study inclusion criteria and all were detectable on SLIC (Fig. 3).

Time to AST in Gram-negative BSIs

The average AST TAT was calculated based on 101 blood culture isolates and 14 different bacterial spp. (Fig. 4). The AST TAT varied with different bacterial spp. using Vitek 2 but was found to be independent of bacterial spp. when using SLIC. A direct comparison of sample runs demonstrated SLIC (1.94±0.02 h) to be more than fivefold quicker than Vitek 2 (10.53±0.46 h).

Comparison of diagnostic timelines using SOC and SLIC in Gram-negative BSI

The impact of SLIC on the diagnostic workflow in the study hospital was significant and enabled the TAT from patient sampling to AST result to be achievable within 29.9±1.05 h. A possible time saving of 23.96±1.47 h was found when compared to SOC with a TAT of 53.8±1.49 h (Fig. 5).

Comparison of AST outcome using SLIC and SOC

The AST outcome of SLIC and SOC was reported for 101 blood cultures against 5 antibiotics and a total of 505 AST results were produced. A categorical agreement of 95.5% (482/505) was achieved between SLIC and SOC (Tables 1 and 2). The classification of susceptible or resistant GNB using SOC compared to SLIC was not found to be significantly different based on McNemar's test ($P>0.05$). All 23 discrepancies between SLIC and SOC were further investigated by BMD. The BMD result agreed with the SLIC AST outcome on 10 out of 23 occasions (43.5%) and agreed with SOC on 13 out of 23 occasions (56.5%).

AMR in Gram-negative BSI

SLIC successfully detected six MDR cases where isolates were resistant to three or more antibiotic classes within 2 h of Gram stain. Additionally, SLIC detected 13 cases where isolates were resistant to two different antibiotic classes. Two of these cases detected by BMD and SLIC were not reported by SOC. AMC resistance was most prevalent compared to all other antibiotics evaluated. SLIC identified 52/55 cases of AMC resistance compared to 49/55 cases using Vitek 2. BMD highlighted that three cases of AMC resistance were missed by SLIC and six by Vitek 2. AMC resistance was most associated with β-lactamase-producing *E. coli* (79.6%) and was detected in 42/43 cases on SLIC and 40/43 cases on Vitek 2. Potential extended β-lactamase-producing organisms were identified on 14 occasions using SLIC. Using SLIC and BMD allowed an additional case of CRO resistance to be detected, which would have otherwise been missed by disc diffusion. SLIC detected 12 GEN-resistant isolates, 1 more than Vitek 2. An additional case of CIP resistance was detected by Vitek 2 compared to SLIC. No MEM-resistant isolates were detected in the study.

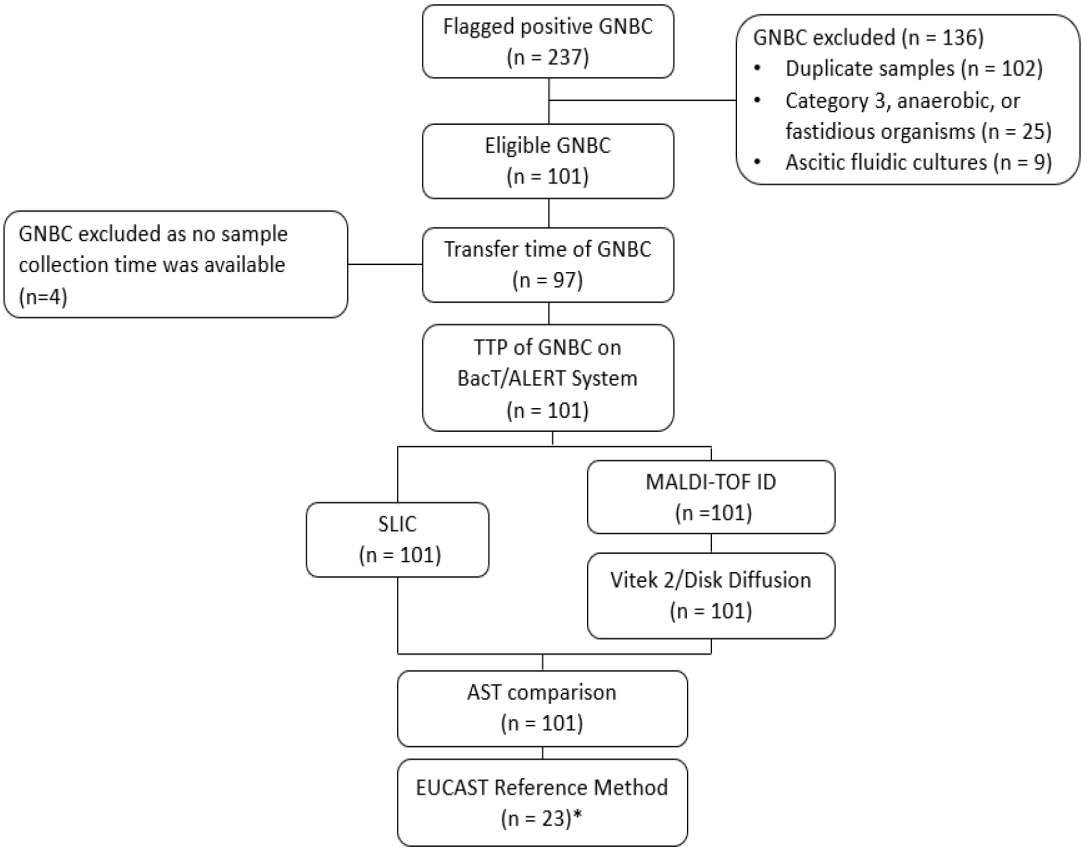

Fig. 3. The number of blood cultures studied and their final disposition. The AST outcome of SLIC and SOC was compared for 101 blood cultures. Excluded were all Gram-negative blood cultures (GNBCs) that could not be examined using Vitek 2, including category 3 organisms and anaerobic and/or fastidious organisms. Polymicrobial blood cultures containing Gram-positive and GN bacteria were excluded following Gram stain. Mixed GN blood cultures were excluded from analysis retrospectively following matrix-assisted laser desorption/ionization time-of-flight ID. Blood cultures that flagged positive and were not processed within 48 h, ascitic fluid cultures and duplicate patient blood cultures from the same bacteraemia episode were also excluded. A full list of excluded blood cultures can be found in Table S2, available in the online version of this article. *A total of 23 AST discrepancies occurred between SLIC and the standard workflow, and these were further investigated using the EUCAST microdilution broth method.

DISCUSSION

Principal findings

Generally, blood cultures are mono-microbial 89–95% of the time, making them an ideal candidate for rAST [16]. Gram staining is a fast and simple method to select appropriate blood culture and enabled rAST to be completed in under 2 h on SLIC and an AST result to be available up to 24 h earlier than SOC.

The SOC workflow utilised the automated AST platform, Vitek 2 – the quickest commercially available high-throughput bacterial ID and AST system. The drawback of this approach is the requirement for a secondary culture to be inoculated from the initial blood culture bottle. This can add over a day to the TAT, meaning in most cases that AST results are available after treatment decisions are made.

A rapid disc diffusion AST (RAST) method has been developed by EUCAST. This method provides a provisional indicator of bacterial susceptibility following a 4, 6 or 8 h incubation using adjusted breakpoints. The early provisional RAST result can be reported to the clinician to guide antimicrobial choice; however, this must be confirmed later by a secondary AST method as the inoculum is not standardised. To date, EUCAST has validated this method for eight bacterial species, and while it is quicker, it remains manual and labour-intensive [17]. In comparison, SLIC has been shown to consistently provide rAST for a range of clinically significant pathogens within half the time of the quickest incubation (4 h) advised when using the EUCAST RAST method and requires as little as 10 min of hands-on time.

Several commercially available and regulatory-approved phenotypic platforms can offer rAST, including the Avantage system (Abbott), ALFRED 60 AST (AliFAX), PhenoTest BC (Accelerate Diagnostics), dRAST (QuantaMatrix), Lifescale Blood ID

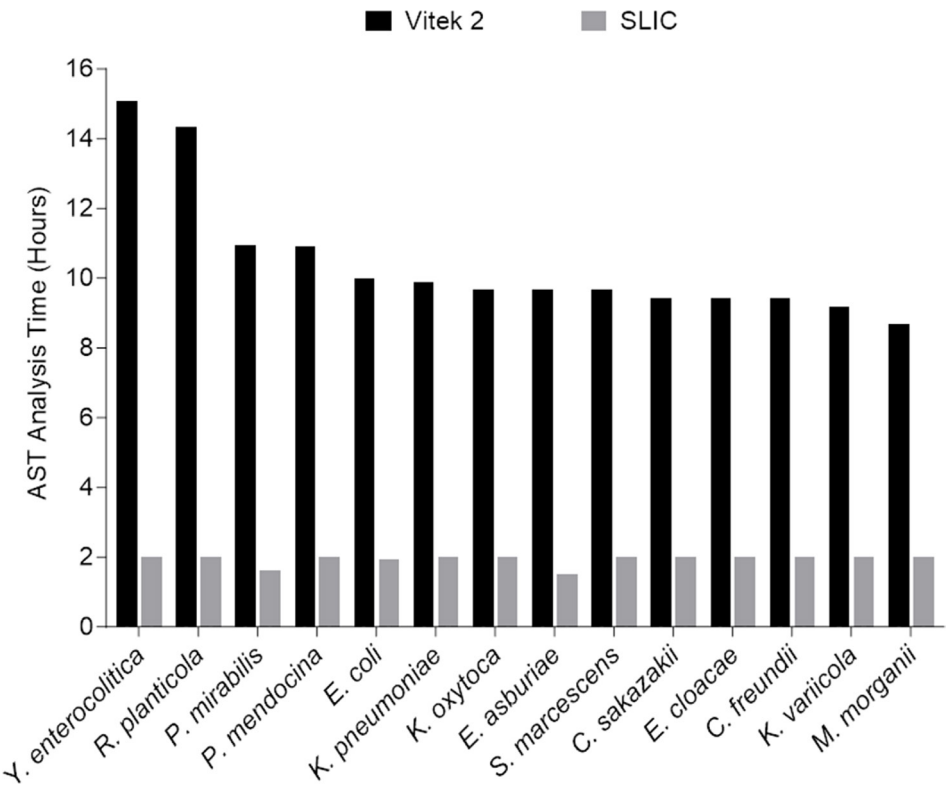

Fig. 4. AST time comparison of Vitek 2 and SLIC. The median AST analysis time for Vitek 2 was 9.67 h (IQR 9.425–10.95) for Gram-negative BSI compared to a median SLIC AST time of 2 h (IQR 2–2).

system (Lifescale), ASTar (Q-linea) and FASTinov (FASTinov S.A.). These rAST platforms can indicate bacterial susceptibility in 1.3–7 h directly from positive blood culture with an AST categorical agreement of 89–98% when compared against a standard AST method [18–24]. However, progress in integrating many of these promising diagnostic technologies into the clinical pathway has been hindered, mainly due to expensive set-up and running costs, logistical challenges, and the technical expertise and time investment required.

Alternatively, molecular multiplex RT-PCR methods can offer a rapid insight into select AMR mechanisms. For example, the MagicPlex Sepsis assay can detect up to 90 sepsis-causing pathogens and 3 resistance genes directly from whole blood in 6 h

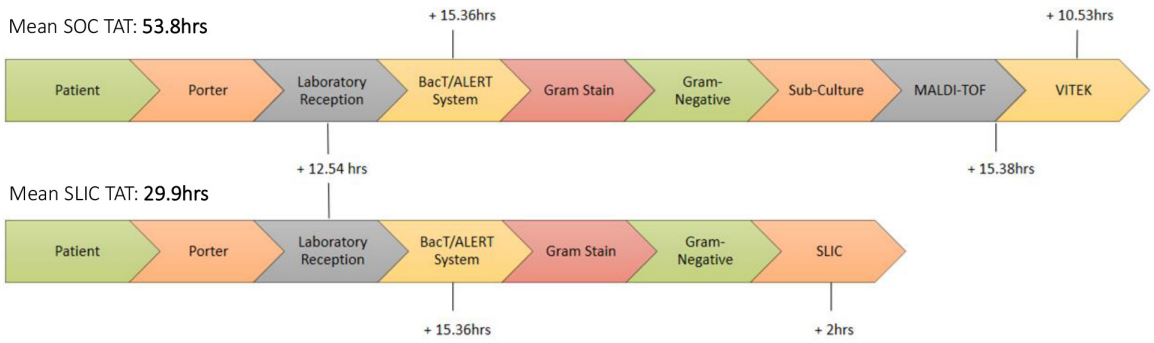

Fig. 5. Comparison of time to AST result using SOC and SLIC. The sample journey from patient to AST report was measured over a 5-month period for 101 patient samples. The time control points included: transfer time (12.54+1.28 h*), BacT/ALERT analysis time (15.36+1.84 h), transfer time to Vitek 2 (15.38+1.49 h), Vitek 2 AST analysis time (10.53+0.46 h) and SLIC AST analysis time (1.94+0.02 h). The SLIC workflow demonstrated a possible time saving of 23.96+1.47 h, a 44.5% reduction to AST result when compared to the conventional method of sub-culture and Vitek 2. The SLIC workflow was calculated based on the direct processing of a positive blood culture following the Gram stain result. *Average transfer time was based on 97 samples as four samples had no collection time.

Table 1. Categorical agreement between SOC and SLIC based on antibiotic. The most frequent discrepancies were associated with amoxicillin–clavulanic acid (AMC) (12) and ceftriaxone (CRO) (7). AMC was associated with three major errors and nine very major errors. CRO was associated with seven major errors. A total of 23 disagreements occurred between SOC and SLIC

Antibiotic	AST by SOC		SLIC AST agreement		%CA
	Susceptible (*n*)	Resistant (*n*)	Agreed (*n*)	Disagreed (*n*)	
CRO	88	13	94	7	93.1
MEM	101	0	101	0	100
CIP	89	12	98	3	97.0
GEN	90	11	100	1	99.0
AMC	52	49	89	12	88.1
Total	420	85	482	23	95.5

[25]. Although molecular methods have the advantage of culture independence and offer the sensitivity and rapidity required, no molecular assay has yet been developed to offer comprehensive AMR coverage.

The combination of rapid molecular bacterial identification methods and universal rAST phenotypic methods such as SLIC offers a promising avenue to bridge the current gap in clinical diagnostics [26]. Across 1010 AST possibilities, the rAST performance of SLIC was highly comparable to SOC, with a categorical agreement of 95.5% (Tables 1 and 2). This is similar to the AST performance reported by other phenotypic rAST methods [18–24]. The affordability, ease of use, high sensitivity and real-time monitoring of antimicrobial response using SLIC are the key advantages over other phenotypic rAST methods and would allow the AST results to be released as and when available without delay [26].

Table 2. Categorical AST agreement between SOC and SLIC differentiated by GNB. SLIC provided rAST in a mean time of 1.94±0.02 h with good agreement with SOC (95.5%). Results based on a five-antibiotic panel including CRO, MEM, CIP, GEN and AMC at EUCAST breakpoints (2019, v 9.0). Note that *Citrobacter freundii*, *Enterobacter cloacae*, *Morganella morganii*, *Pseudomonas mendocina*, *Serratia marcescens* and *Yersinia enterocolitica* are intrinsically resistant to AMC

Organism group	Total tested	No. of ABC*	CA	%CA	Susceptible by SOC	Resistant by SOC	SLIC Vmj	SLIC Maj
Citrobacter freundii	1	5	5	100	2	3	0	0
Cronobacter sakazakii	2	10	10	100	10	0	0	0
Enterobacter asburiae	1	5	5	100	3	2	0	0
Enterobacter cloacae	2	10	10	100	7	3	0	0
Escherichia coli	78	390	372	96.7	322	68	5	13
Klebsiella oxytoca	1	5	4	80	5	0	0	0
Klebsiella pneumoniae	4	20	17	85	20	0	0	3
Klebsiella variicola	3	15	15	100	14	1	0	0
Morganella morganii	1	5	5	100	4	1	0	0
Proteus mirabilis	4	20	20	100	18	2	0	0
Pseudomonas mendocina	1	5	5	100	4	1	0	0
Raoultella planticola	1	5	5	100	5	0	0	1
Serratia marcescens	1	5	4	80	3	2	1	0
Yersinia enterocolitica	1	5	5	100	3	2	0	0
Total	101	505	482	95.5	420	85	6	17
Error percentage							7.1%	4.1%

*Antimicrobial–bacterial combinations.

The high national and international prevalence of AMR emphasises the need to implement rAST to ensure that the correct antibiotic is prescribed in the early stages of BSI [3]. The importance of timely effective therapy was first documented in 1999 by Kollef and has since been demonstrated by numerous other studies [8, 9, 27–31]. All agree that early indication of AST lowers the risk of inappropriate initial antibiotic treatment, which has been associated with significantly poorer clinical outcomes, including higher mortality rates, prolonged hospital stays and recurrence of infections.

The unpredictability of bacterial susceptibility in BSI was highlighted in this study, with AMR detected in 59.4% of blood culture isolates across four different antibiotic classes. Rapid screening of five antibiotic classes using SLIC ensured appropriate coverage for the most frequent origins of Gram-negative BSI – urogenital, gastrointestinal, respiratory and central nervous system [32]. All MDR cases were accurately detected on SLIC and consistently available within a 2 h time frame. Patients with an MDR infection tend to have a poorer prognosis, with a higher risk of treatment failure, adverse outcomes and longer hospital stays [4, 33]. A rAST screen using SLIC would most likely have a profound effect on the management and care of these patients with the ability to review and rationalise antimicrobial therapy up to 24 h earlier. The opportunity to apply evidence-based practice early would also rationalise the use of, and exposure to, expensive and broad-spectrum antibiotics, reduce the risk of *Clostridium difficile* infection, antimicrobial toxicity and AMR development against first-line antibiotics. Additionally, SLIC could be used to assist infection prevention and control teams in implementing measures to reduce transmission of MDR infections, such as appropriate patient placement.

Limitations

This proof-of-concept study was conducted in a single hospital over a 5-month period, which limited the number of samples and the diversity of Gram-negative organisms and associated AMR profiles available to test. *E. coli* is the leading cause of Gram-negative BSIs, and this was well represented by the study [5]. In contrast, the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species) were underrepresented. Promisingly, a pilot study demonstrated that rAST can be performed accurately on the ESKAPE pathogens and prevalent Gram-positive BSI pathogens from simulated blood culture in a similar time frame of 100 min on SLIC (Fig. S1). However, larger clinical studies are needed to further evaluate SLIC.

Ideally, the BMD method would be performed for all samples included in the study to ascertain AST accuracy, but this was not possible due to funding and timing constraints. Instead, the study focused on a direct comparison of the categorical agreement of SOC and SLIC, with only discrepant results further investigated using the BMD method. This highlighted that of the 23 categorical disagreements, the BMD results agreed with SLIC on 10 occasions and with Vitek 2 on 13 occasions. It is fully appreciated that each AST method will have its own limitations and that concordant results between SOC and SLIC may not necessarily agree with BMD. Therefore, comparative results should be interpreted with this in mind to avoid overestimation of performance.

Evaluating the AST performance of SLIC may also have been strengthened by using a molecular method to confirm the presence of AMR genes. It is appreciated that phenotypic AST methods do not account for the risk of genetic resistance, where in some instances an AMR gene may be present but not expressed, and this is an important consideration in understanding host response.

Future work

One of the challenges of performing direct AST is often selecting antibiotics and breakpoints without confirmation of bacterial identification. Based on the fact that Enterobacterales are responsible for most Gram-negative BSIs, the antibiotic concentrations used in the study were informed by EUCAST breakpoints for this bacterial order. This was appropriate for all but one isolate in the study – *Pseudomonas mendocina*. Rapid bacterial ID would be hugely beneficial in streamlining rAST into the diagnostic workflow. This would increase the clinical utility of SLIC by enabling a larger BSI pathogen coverage, earlier optimiation of the AST panel and growth conditions for fastidious organisms such as *Haemophilus influenzae*.

The AST discrepancies between SLIC and Vitek 2 are likely reflective of the differences in AST methods and data analysis models. Most rAST discrepancies (19/23) using SLIC were associated with CRO (7) and AMC (12). Misinterpretation of CRO susceptibility is likely due to bacterial morphological plasticity, which is a phenomenon that causes bacteria to undergo morphological changes in response to stress [34]. This causes difficulty in differentiating between scatter patterns caused by normal growth and induced morphological changes. False-positive rAST results were equally associated with AMC. It is known that the inoculum effect is commonly associated with beta-lactam antibiotics. This is postulated to occur when a higher than standardised bacterial inoculum causes a reduction in antimicrobial concentration reaching individual cells, enabling pre-existing resistant populations to thrive, enabling the stationary growth phase to be reached earlier, and/or reducing the expression of antimicrobial targets such as the penicillin-binding proteins [35]. This is hypothesised to be one of the reasons behind the false positives observed in the study. To prevent this reoccurring, improved standardisation of bacterial inoculum and the use of freeze-dried antibiotics to circumvent antimicrobial degradation will be explored in future studies.

SLIC provides an actionable AST result rapidly, but challenges to implementing SLIC in a diagnostic laboratory remain. The main challenge is the significant time attributed to the pre-analysis stage. The 4 h target time frame between sample collection and laboratory receipt was possible in only 25.8% of cases. This time void can limit the benefit of a new diagnostic platform, even if the new method is proven to be superior to existing methods. The study by Weinbren *et al.* [36] demonstrated that key interventions, such as moving the automated blood culture analyser to blood sciences, enabling blood cultures to be loaded 24 h a day, replacing glass bottles with plastic bottles to allow samples to be transferred via the vacuum system and providing education to clinical staff, enabled 95% of blood cultures to be loaded within 2 h [36]. While it is appreciated that not all these interventions may be applicable or possible in every diagnostic laboratory, it demonstrates that even simple reminders of the importance of the timeliness of blood culture may make a significant impact going forward.

Conclusions

SLIC offers accurate rAST for Gram-negative BSI pathogens in a clinical setting, expediting TATs over current standard AST methods. Ease of use, affordability and ability to monitor rAST in real time further support the clinical utility of SLIC. SLIC is an attractive alternative technology for use in microbiological diagnostics, which could potentially change the outlook for BSI patients by detecting antimicrobial-resistant infections a day earlier. A larger multicentre cohort study is warranted to thoroughly evaluate the rAST performance of SLIC on a wider range of BSI organisms in a clinical setting and the potential impact on patient outcomes.

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Author contributions
S.H.G., R.H. and B.J.P. were responsible for the conceptualization of the study, funding acquisition and supervision. All authors contributed to the investigation of this study. K.F. was responsible for data curation, formal analysis, methodology, visualization of data and writing the original draft. Study data were validated by S.H.G., R.H. and K.F. All authors contributed to the review and editing of the report. All authors are able to access all relevant data and accept responsibility for the submission of the study for publication.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The study was reviewed and approved by the ethics committee at the University of St Andrews and by NHS Tayside Health Board. The study used anonymized surplus patient samples. Patients were not recruited and nor did they participate in the study.

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