**Impact of rapid susceptibility testing on antimicrobial therapy and clinical outcomes in Gram-negative blood stream infections**

Vanesa ANTON-VAZQUEZ 1\*, Cristina SUAREZ 1 , Timothy PLANCHE 1,2

1 Institute of Infection and Immunity, St. George’s University of London, London, UK.

2 Department of Medical Microbiology, Southwest London Pathology, St. George’s Hospital, London, UK; Infection Care Group, St George’s University Hospitals NHS Foundation Trust, London, UK.

Running title

Impact of rapid AST in blood stream infections

Corresponding author: Vanesa Anton-Vazquez

[v.anton-vazquez@nhs.net](mailto:v.anton-vazquez@nhs.net) ; [vantonva@sgul.ac.uk](file:///C:\\Users\\vanesaantonvazquez\\iCloud%20Drive%20(Archive)\\Desktop\\vantonva@sgul.ac.uk))**Abstract**

**Background:** Rapid antimicrobial susceptibility testing (rAST) has the potential to improve care of blood stream infections. The aim of this service evaluation is to assess the impact of rAST on antimicrobial therapy and clinical outcomes in patients with Gram-negative blood stream infection.

**Materials/methods:** A prospective service-evaluation was conducted from March 2018 to December 2018. Rapid AST system (Alfred 60AST) was run Monday-Friday before midday and results were communicated to clinicians in the same day of positive blood culture, with subsequent conventional AST performed. Times-to-antibiotic therapy and clinical outcomes were compared between rapid and conventional AST.

**Results:** 191 patients with Gram negative bacteraemia were included, 93 in the rapid and 98 in the conventional group. Aminoglycoside combination therapy was stopped earlier in the rapid group 32h (0-795) versus 54h (4-216), p=0.002. The median time to optimal antibiotic based on AST results was significantly shorter than that in the conventional group 50h (10-339) versus 69.5 h (20-872), p = 0.034. In the subgroup of patients on ineffective empirical antibiotic, time to effective antibiotic was shorter in the rapid group 39.5h (32-97) versus 57h (49-83), p= 0.036. No differences were found in 28-day mortality or length of stay.

**Conclusions:** Rapid susceptibility testing resulted in faster discontinuation of aminoglycosides and a shorter time to start effective and optimal antibiotic when compared to conventional AST results. Rapid AST has potential clinical benefits in point to the need for larger future studies in areas of high antibiotic resistance for future studies.

Word count (abstract): 245

Word count (manuscript): 3477

## **Introduction**

The outcome of patients with sepsis is worse if antibiotics are not administered promptly. 1-5 6 In patients with Gram-negative blood stream infection (BSI), inappropriate empiric antimicrobial therapy has been associated with increased mortality, particularly in settings with high prevalence of multidrug resistance. 7 8 9 10

Clinical microbiology laboratories play a key role in the diagnosis of BSIs, as the definitive decision for what antibiotic to use is based on *in-vitro* susceptibility results from bacteria after they have been grown in routine culture. It may take several days to obtain antimicrobial susceptibility results from blood cultures, which can have a profound effect on patient care pathways. One of the most important delays in obtaining antimicrobial susceptibility test (AST) results is the time taken for *in-vitro* susceptibility testing after an organism has been identified in a blood culture, using conventional techniques this may take 36 hours or more. 11

The delay in routine AST results and the fact that only around 30% of patients with sepsis have a positive blood culture 12 means that patients start an empiric choice of antibiotics before AST results are available. The choice of antibiotic therapy is based on the knowledge results of antibiotic susceptibility tests of local isolates. This empiric choice of antibiotic tends to be broad spectrum to adequately treat over 90% of historic isolates. Once the results of AST are known antibiotic therapy may then be switched to a targeted narrow-spectrum antibiotic. This so called “start smart then focus” policy tends to mean that patients are treated with broad spectrum antibiotics for many days until the results of AST are available. As the use of broad-spectrum antibiotics is associated with the spread of antibiotic resistant organisms delays in AST results may lead to increases in antibiotic resistance. 13

Novel rapid antimicrobial susceptibility tests (rAST) have the potential to reduce the time to result, allowing doctors to switch patients onto appropriate antibiotic therapy more quickly than traditional microbiological methods. rAST may also reduce reliance upon broad-spectrum antibiotics and reduce spread of antibiotic resistance. However, actual patient benefit has not yet been objectively shown in clinical outcome-based studies. 14

Over the last decade, many new automated technologies have been introduced in microbiology with the aim of providing the AST results to significantly reduce the time to appropriate or effective antimicrobial therapy. Recently developed rAST systems that use different technologies to provide identification and antimicrobial susceptibility results in as short as 4-7 hours after blood culture positivity. 15-18 Although there is an agreed need for improved speed of diagnosis, there is no clear understanding of what is the impact this will have or of how this will result in improvement in patient pathways or outcomes.14 In this study, we aimed to identify the effects of a rapid antimicrobial susceptibility system after its introduction into routine laboratory practice on process, antimicrobial and clinical outcomes in patients with Gram-negative blood stream infections.

## **Material and methods**

#### **Study site and patients**

A prospective service-evaluation study after the introduction of the Alfred 60 AST (Alifax, Italy) into routine laboratory practice was conducted at St. George’s University Hospitals NHS Foundation Trust, London, a tertiary teaching hospital in south London with around a thousand beds and sixty thousand hospital admissions per year.

Consecutive patients of any age who had at least one blood culture with a pure growth of a Gram-negative bacteria on microscopy, between March 2018 and December 2018 were included. Routine blood cultures from all clinical wards and the emergency department of the hospital were included in this study. Only the first BSI episode was evaluated for each patient. Patients were excluded if: i) more than one organism grew in the blood culture (polymicrobial sample), ii) anaerobic gram-negative organism was isolated, iii) clinically not relevant isolates (e.g. environmental contaminant not causing clinical infection), iv) the patient died before the index blood culture became positive.

**Data collection**

Data collected was transferred to a specific case report form (CRF) and then to a purpose-built anonimysed web-based database. The infection specialists managed those patients routinely as per their local protocols. The research fellow liaised daily with the infection specialist or the designated person to identify positive blood cultures to be included in the study, without interfering the conventional and routinely workflow and current activity of the department.

#### **Laboratory Methods and workflow**

The laboratory is open 24 hours a day, blood cultures samples collected on the wards were inoculated into blood culture bottles and incubated on the BacTec automated culture system (Becton Dickinson, USA) for a maximum 5 days. When a bottle was indicated as positive on the BacTec system it was removed and inoculated immediately on blood agar and a Gram stain made. The blood agar plate was incubated for 4 hours to allow the early growth to be used for matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry **(**MALDI-TOF MS) (Brucker, Germany) identification of species of organism.

Routine laboratory practice was as follows: Gram staining was performed on positive blood culture bottles from 6am to 10pm daily. No Gram-staining of positive BC bottles was performed after 10pm at night. Blood cultures found positive after 10 pm were analysed and reported the following morning after 6am, due to limited laboratory staff resources and limited communication of results during night hours.

If a Gram-negative isolate was identified, the specimen simultaneously set up for routine antimicrobial susceptibility testing (BD Phoenix) with or without rapid testing (Alfred 60AST). Only positive blood culture bottles from Monday to Friday “in-hours” from 8 am to 10 pm were included for each arm. Blood culture bottles flagging positive after 10 pm or weekends were not included in the study. Patients included in the rapid testing group (Alfred 60AST) had blood cultures flagging positive from 8 am to 12 pm from Monday to Friday, in addition to all other normal laboratory diagnostic procedures. Patients included in the conventional testing group (BD Phoenix) had blood cultures flagging positive from 12 pm to 10 pm from Monday to Friday. Bacterial species was identified by MALDI-TOF MS (Bruker, Germany) from a blood agar plate incubated for 4 hours. Patient care remained unaffected by the service review (Figure 1).

#### **The rapid system**

Alfred 60AST is a CE mark validated system for antimicrobial susceptibility directly from positive blood cultures and has been verified in our clinical laboratory.19 The Alfred 60 AST was performed according to the manufacturer’s instructions with 30µL of the blood culture sample inoculated into an enrichment broth and loaded onto the instrument. Each vial of antibiotic contains 45mg of a preparation of lyophilized antibiotic that requires to be dissolved in 2mL of regenerating solution to give a concentration of antibiotic at a specified breakpoint. Some antibiotics, including amikacin, piperacillin-tazobactam and meropenem, require different distinct antibiotic vials for testing Enterobacteriaceae and *Pseudomonas Spp.* As the identity of organism was only known after the Alfred 60 AST system was set up, a combined panel of antibiotics with both concentrations was used.

#### **Comparator AST technique**

The BD Phoenix™ automated susceptibility testing system (BD Diagnostics, Sparks, MD) (software version 5.02H/4.11B) was used as the routine laboratory method according to the manufacturer instructions using cartridge NMIC-417 for Gram-negative isolates.

#### **Interpretation and Communication of results**

Gram-staining results were communicated to the medical team as soon as they were available, between 8am and 10pm. rAST along with identification results of the organism were electronically reported and actively communicated on telephone or in person by a clinical microbiologist to the attending clinician on the same working day of positive blood culture. Susceptibility results obtained by routine laboratory diagnostic procedures (Phoenix BD) were reported and communicated when they were available, usually on the next day.

#### **Empiric Antibiotic therapy**

The antibiotic treatment policy for the hospital remained unchanged. The routine policy for the hospital depended upon the presumed source of infection and previous known microbiology results. The policy was to treat sepsis of presumed urinary sepsis and sepsis of unknown origin with a combination therapy with an aminoglycoside and a beta-lactam antibiotic, specifically gentamicin and amoxicillin/clavulanate.

#### **Definitions of variables**

#### **Antimicrobial outcomes**

Antibiotic therapy was defined as effective if the isolate was susceptible *in vitro* to that antibiotic. Antibiotic therapy was optimal if two criteria were met: 1) the pathogen was susceptible to the antibiotic *in vitro*, and 2) the spectrum was the narrowest based on a ranking chart from narrow spectrum (rank 1) to very broad spectrum (rank 5) based on previously published antibiotic rankings. 20 (Table S1).

Escalation or addition or starting an additional antibiotic or switch from oral to intravenous was defined as change from narrow to broad spectrum antibiotics. De-escalation or discontinuation was defined as change from broad to narrow spectrum antibiotics or discontinuation of an antibiotic or switch from intravenous to oral.

Time to antibiotics was defined as the time in hours from blood culture collection to start, discontinue or change antibiotic, according to the above definitions.Mortality was defined as all-cause 28-days mortality, after date of positive blood culture) and lengthof stay was time to discharge from hospital after positive blood culture in days

##### **Laboratory process outcomes**

Time to antimicrobial susceptibility results released by the laboratory was defined as the time in hours from blood culture collection to when the laboratory made the results available to a clinical microbiologist. Time to antimicrobial susceptibility results communication to clinicians was defined as the time in hours from blood culture collection to communication of results to the attending clinical team and clinical advice.

#### **Demographics variables definitions**

Antibiotic susceptibilities were checked against an automated antimicrobial susceptibility system (BD Phoenix", BD Diagnostics). Charlson comorbidity index was measured and calculated for adult and paediatric population at the onset of blood stream infection.21 National Early Warning System (NEWS) Score assessed Severity of illness, which has been validated across hospitals in the UK. 22

#### **Laboratory data**

Categorial agreement was defined as agreement between the Phoenix and Alfred AST60 systems for an antibiotic by S/I/R (susceptible/intermediate/resistant) categories. For this service evaluation, the Phoenix system was counted as a system for reference and discrepancies were classified as follows: very major (VM, reported by Alfred AST60 susceptible when resistant) , major (M, reported resistant when susceptible) or minor (m, reported intermediate when susceptible or resistant). 23

#### **Statistical analysis**

All dichotomous variables were analysed by Fisher’s exact test. Normally distributed continuous variables were analysed using the 2-tailed Student’s *t* test. Continuous variables were analysed using the Mann-Whitney U test. A *P* value of <0.05 was considered statistically significant. Univariate and multivariate analysis was performed to evaluate prediction of dependent variables by the independent ones. Factors with a p value 0.3 based on univariate modelling were included in a multivariate logistic regression analysis. Survival was estimated via the Kaplan-Meier test. Data were assessed using SPSS version 25

**Ethical approval**

The Joint Research office in St. George’s on 14th December 2017 confirmed this as a service evaluation, not requiring ethical review, according to the UK Policy Framework for Health and Social Care Research v3.2 October 2017. No personal information was stored in the study database. Samples were collected as part of standard care, which was unchanged during the service evaluation.

**Results**

There were 211 episodes of Gram-negative bacteraemia during the study period. Nine episodes were excluded, of which three were polymicrobial samples, three anaerobes, two recurrent bacteraemia episodes and one clinically non-significant isolate (*Prevotella salivae*). 202 episodes of Gram-negative bacteraemia were included in the final service evaluation. A total of 93 patients with Gram-negative bacteraemia received a rapid susceptibility testing and 98 patients had a conventional susceptibility testing done (Figure 2).

**Characteristics of patients**

Patient characteristics and demographics are shown in (Table 1).The groups were well balanced in their epidemiological and clinical characteristics. *Escherichia coli* was the most frequent organism, responsible for 67% and 57% in the rapid and conventional group, followed by *Klebsiella* spp. responsible for 13% and 14% in the rapid and conventional group, respectively. The two groups did not differ statistically with regard to co-morbidity, nosocomial acquisition of bacteraemia, clinical severity, source of infection.

**Performance of Alfred 60 AST**

In the rapid group, a valid antimicrobial susceptibility result was yielded in 97/102 cases. Overall CA for AST for the rapid system Alfred 60AST, was 94% (666/ 709) (Table 2).

**Time to results**

The overall median (range) time-to-positivity of the initial blood culture in the BacTec automated culture was 20.48 hours (2-108) and was not significantly different between groups (rapid 19.16 h (2-106) versus conventional 18.7 h (2-110), p= 0.37). The median time from blood culture collection to communication of antimicrobial susceptibility testing (AST) results to the clinical team was significantly reduced from 55.2 h (33-145) in the conventional group and 54.7 h (33-145) as part of the parallel assignment to conventional AST in the rapid group to 33.1 h (17-123) in the rapid group (p<0.001).

**Antimicrobial outcomes**

Initial empirical effective antimicrobial therapy was administered in 86 (92%) patients in the rapid group and 93 (95%) in the conventional group (p=0.861) before the results of antibiotic susceptibility were known. No differences were found in median time to effective antimicrobial between rapid and conventional group, 3 h (0 -97) versus 4 h (0 -120) respectively, p= 0.490 (Table 4). In the subgroup of patents on ineffective antimicrobial, effective treatment based on AST results was started 17.5 hours earlier in the rapid group compared with the conventional group (n=6; 39.5 h (32-97) versus n=-5; 57 h (49-83), p= 0.036) (Table 4).

Escalation of non-aminoglycoside antibiotic co-therapy on AST results was shorter in the rapid group compared with the conventional group (n= 34; 41.7h (21-103) versus n=15; 66.9h (46-121); p=0.010) (Table 4). Kaplan-Meier curves showed a statistically significant shortening in time to escalate antibiotic at 72 hours in the rapid group compared with conventional group (Figure 3).

Combination therapy with aminoglycosides was stopped based on AST results in 57% (40/70) patients in the rapid group and in 37% (24/64) in the conventional group, *p*= 0.038 (Table 3). In this subgroup of patients, aminoglycosides were discontinued earlier in the rapid group; 48.8 h (0-795) compared with 53.7h (4-216) in the conventional group, p=0.017(Table 4). Kaplan-Meier curves showed a statistically significant shortening in time to stop aminoglycosides at 48 and 72 hours (Figure 3). The proportion of patients that received 2 doses of aminoglycosides based on AST results, was significantly lower in the rapid group compared with the conventional group (26/40 (65%) versus 23/24 (96%); p <0.005) (Table S2). Rapid AST was a predictor for discontinuation of aminoglycosides at 48 hours (OR 2.3; 95% [CI 1.1- 4.80], p=0.027) and to receive only one dose of aminoglycosides (OR 3.24; 95% [1.47 – 6.98], p= 0.003) in binary logistic regression (Table 5).

Optimal antimicrobial treatment was eventually prescribed in 63 (68%) patients in the rapid group and in 66 (67%) in the conventional group, p=1. In the subgroup of patients who were on non-optimal antimicrobial, antibiotic changes were made following AST results in 59 (93%) patients in the rapid group versus 58 (88%) patients in the conventional group (p=0.876). The time to optimal antibiotic based on AST results was significantly shorter than that in the conventional group (n= 59; 50h (10-339) versus n=58; 69.5 h (20-872), p = 0.034) (Table 4). Kaplan-Meier analysis showed a statistically significant shortening in time to start optimal antibiotic at 72 hours in the rapid group compared with conventional group (Figure 3).

In the subgroup of patients in whom optimal antibiotic change was achieved based on AST results, the most common antimicrobial change in the rapid group was escalation of non-aminoglycoside antimicrobial (n, 12 (20%) versus n, 3 (5%) p= 0.024). This contrasts with the conventional group, where de-escalation of non-aminoglycoside antibiotic was the most common antimicrobial change (n, 23 (39%) versus n, 39 (67%), p=0.003) (Table 3). Overall, time to antimicrobial de-escalation of the non-aminoglycoside antibiotic was found shorter in the conventional group 97.8 h (8-501) compared with the rapid group 125.2h (24-632), although it was non-statistically significant p= 0.071. No differences were noted in time to stop all antibiotics 370.2h (31-1126) rapid versus 335.6h (50-1834) conventional, p= 0.219 (Table 4).

The number of missed opportunities to de-escalate antibiotics when AST were available accounted for 30 (34%) in the rapid group, compared to 32 (36%) in the conventional group. The main reasons to not change antibiotics were, “clinical condition” 24 (80%) rapid versus 24 (75%) conventional, p=0.764 and “awaiting conventional AST results” in 5 (17%) cases in the rapid group, p= 0.002. (Table S3).

No differences were found in 28-day mortality (rapid n=7 (8%) versus conventional n=13 (13%), p= 0.24) or median length of hospital stay (11 days (0-47) in the rapid versus 10.5 days (0-71) in the conventional group, p=0.84) (Table 3).

**Discussion**

Rapid antimicrobial susceptibility testing in Gram-negative BSI resulted in earlier discontinuation of aminoglycoside therapy, earlier initiation of effective antibiotic in those patients on ineffective empiric antibiotic, shorter time to optimal antibiotic and shorter time to escalation of non-aminoglycoside antibiotic. In line with this, a reduction in time to first effective antibiotic was also observed by *Kim et al*. 24 25 also found the mean time to optimal antibiotic treatment was significantly shorter in the rapid phenotypic group compared with the conventional group.

Our findings show that rapid AST leads to antibiotic changes being effected almost 24 hours earlier. This significant reduction in the time to results, however had no detectable effects on clinical outcomes in a study of this size. In line with this, no differences in patient outcomes were observed by other authors. 24 26 25 Nevertheless, we observed that the mortality rate was double in the conventional group compared with the rapid group, although not statistically significant. This is interesting and could indicate that larger sample size would be needed to demonstrate differences in mortality. As the impact of a day of additional antibiotic therapy on toxicity or antibiotic resistance is likely to be small, large studies would be needed to detect differences in factors such as carriage of resistant organisms or to assess potential toxicity derived from aminoglycoside treatment.

In our study, the proportion of patients on effective antibiotic was as high as 94%. This contrasts with up to 40% of ineffective empiric antimicrobial therapy reported by various authors. 27 5 28 29,30 A reason for this, might be the low antibiotic resistance rates in the UK compared to other study sites. Antibiotic treatment was in accordance with our local epidemiology and antimicrobial empiric guidelines for Gram-negative bacteraemia, which are based on aminoglycosides combination therapy. As a result, extrapolation of findings in different populations may not be possible and different settings with different antimicrobial resistance prevalence could have different results on antimicrobial and clinical outcomes.

This service evaluation was prospective and benefitted from a comparator group of patients with similar characteristics to patients in the rapid group. The main difference between the two groups was that patients in the rapid group had antimicrobial susceptibility results on the same day blood culture bottles flagged positive, whilst in the control group, the susceptibility results were available on the following day. In contrast, earlier observational studies, were limited by their retrospective designs, use of historical controls and failure to match patients by disease severity. 31 32 33 Moreover, in keeping with routine practice in the local laboratory, microbiology results were directly communicated to the medical team by clinical microbiologists by telephone. However, our study presents several limitations. Alfred 60 AST system is unable to perform susceptibility results from polymicrobial samples and relies on Gram stain results to set up the correct panel, which is a common problem of new rapid susceptibility systems. 34 The non-random nature of the study is also a potential bias, whereby laboratory practice dictated that rapid testing was only performed on samples becoming positive at certain hours. However, given the median then time taken for the conventional Phoenix to provide results after a positive culture was 20 hours then blood cultures in the rapid group would have conventional results available between 6pm and 8 am and the conventional group available between 8am and 6pm weekdays. Given the laboratory read the conventional Phoenix AST from 9 am to 9 pm the estimates here may be an underestimate of the relative time to results compared to conventional testing.

We observed that there were more missed opportunities to de-escalate non-aminoglycoside antibiotic than to escalate antibiotic. This observation in our study, leads to support a “targeted” approach, in which only a limited range of key antimicrobials may serve as an early warning system to predict resistance as an early step being incorporated into the routine blood culture pathway before the final AST results are available.

In conclusion, we showed that rapid susceptibility results provided along with identification results in Gram-negative bloodstream infections had a positive antimicrobial outcome by shortening the time to aminoglycoside discontinuation, time to start effective and optimal antibiotic when compared to conventional AST results. The fact that we showed no impact on clinical outcomes including mortality or length of hospitalisation was to be expected given the size of the study, with only eleven patients not on effective antibiotic therapy at the time of AST results. This demonstrates value to the antimicrobial management of Gram-negative bacteraemia, particularly when combination therapy with aminoglycosides is used and points to the need for larger future studies in areas of high antibiotic resistance for future studies.

**Acknowledgements**

We thank the team of laboratory technicians and the clinical microbiology and infection team at St. George’s University hospital NHS, London, for their valuable work and technical assistance.

**Funding**

This work was supported by funding from National Institute for Health Research (NIHR) Collaboration for Leadership in Applied Health Research and Care (CLAHRC) South London, London, UK (12951-20).

The funding body had no role in study design, data collection, analysis, interpretation of data, writing the manuscript and decision to publish.

**Transparency declarations**

None to declare

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**Table 1*.*** Baseline patient characteristics. MEWS: Modified Early Warning Score. ESBL: Extended spectrum beta-lactamase. CRP: C-reactive protein. ICU: Intensive Care Unit. AST: Antimicrobial Susceptibility Testing.

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| --- | --- | --- | --- |
|  | Rapid  n = 93 | Conventional  n = 98 | p-value |
| Female | 43 (46.2%) | 49 (50.5%) | 0.565 |
| Age in years; mean (SD) | 65.4 (SD 20) | 63.7 (SD 25) | 0.607 |
| Co-morbidity (at least one)  Charlson index score; mean (SD) | 88 (94.6%)  5.7 (SD 2.8) | 90 (91.8%)  5.6 (SD 2.9) | 0.569  0.665 |
| Organism  *Escherichia coli*  *Klebsiella* spp.  *Proteus* spp.  *Pseudomonas* spp.  *Enterobacter* spp.  *Citrobacter* spp.  *Serratia* spp  Others | 62 (67%)  13 (13%)  2 (2%)  2 (2%)  5 (5%)  2 (2%)  3 (3%)  4 (%) | 56 (57%)  14 (14%)  4 (4%)  8 (8%)  9 (9%)  1 (1%)  1 (1%)  8 (8%) | 0.184  0.886  0.683  0.215  0.408  0.613  0.358  0.721 |
| Resistance  1 antibiotic group  2 or more antibiotic groups | 78 (84%)  15 (15%) | 70 (71%)  28 (28%) | 0.056  0.056 |
| Resistance pattern  Penicillinases  ESBL  Carbapenemases  AmpC  Quinolones  Aminoglycosides  Colistin | 48 (52%)  16 (17%)  1 (1%)  0 (0%)  16 (17%)  3 (3%)  1 (1%) | (49%)  12 (12%)  5 (5%)  3 (3%)  17 (17%)    9 (9%)  7 (7%) | 0.773  0.414  0.212  0.247  0.566  0.135  0.065 |
| Acquisition  Community-acquired  Healthcare-associated  Nosocomial | 30 (32%)  43 (46%)  20 (22%) | 31 (32%)  41 (42%)  26 (27%) | 1  0.562  0.499 |
| Specialty type  Medical  Surgical | 60 (65%)  33 (36%) | 73 (74%)  25 (26%) | 0.203  0.203 |
| Source  Urinary  Abdominal/ biliary  Respiratory  Endovascular  Skin/ soft tissues  Gynecology  Unclear source | 48 (52%)  21 (22%)  4 (4%)  9 (9%)  2 (2%)  2 (2%)  7 (7%) | 49 (50%)  19 (19%)  7 (7%)  10 (10%)  1 (1%)  2 (2%)  10 (10%) | 0.885  0.480  0.538  1  0.613  1  0.615 |
| Clinical severity (at the time of inclusion)  NEWS score > 4  Pitt score > = 2  CRP (mg/dl) | 36 (39%)  35 (38%)  134 (3 – 507) | 43 (44%)  49 (50%)  103.5 (2 – 444) | 0.735  0.101  0.023 |
| Admitted to ICU at the time of inclusion | 3 (3%) | 8 (8%) | 0.215 |
| Source control | 45 (48%) | 45 (46%) | 0.773 |
| Empirical antibiotic  Monotherapy    Beta-lactam (no carbapenem)    Carbapenem  Quinolones  Combination therapy    Amoxicillin/clavulanate + aminoglycoside  Cephalosporin + aminoglycoside  Piperacillin/tazobactam + aminoglycoside  Carbapenem + aminoglycoside  Quinolone + amoniglycoside | 93 (100%)  23 (25%)  19 (20%)  4 (4%)  0 (0%)  70 (76%)  25 (27%)  4 (4%)  19 (20%)  19 (20%)  3 (3%) | 98 (100%)  34 (35%)  23 (23%)  7 (7%)  4 (4%)  64 (65%)  25 (25%)  3 (3%)  12 (12%)  17 (17%)  7 (7%) | 0.606  0.155  0.814  0.627  0.415  0.112  0.995  0.956  0.268  0.789  0.407 |

**Table 2*.*** AST results of the Alfred 60 AST™ system compared to BD Phoenix™ system for 93 Gram-negative organisms included in the study. Column 2 refers to the number of tests for which there was agreement between both tests. S=susceptible, I = intermediate, R=resistant, CA= Categorical agreement. Abbreviations: m = Minor error; M= Major error; VM= Very Major error.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | No. of category agreements | | No. of discrepancies | | | |
|  | CA Total | CA (%) | Tot. | m | M | VM |
| Gram-negative antimicrobials | | | | |  | |
| Ampicillin | 91/ 93 | 98% | 2 | 2 | 0 | 0 |
| Amikacin | 86/ 93 | 93% | 7 | 5 | 2 | 0 |
| Ciprofloxacin | 87/ 93 | 94% | 6 | 5 | 1 | 0 |
| Ceftriaxone | 80/ 93 | 86% | 13 | 10 | 3 | 0 |
| Gentamicin | 83/ 93 | 89% | 10 | 9 | 0 | 1 |
| Piperacillin/Tazobactam | 92/ 93 | 99% | 1 | 1 | 0 | 0 |
| Meropenem | 91/ 93 | 98% | 2 | 1 | 1 | 0 |
| Amoxicillin/ Clavulanate | 56/ 58 | 97% | 2 | 2 | 0 | 0 |
| Overall agreement | 666/ 709 | **94%** | 43 | 35 | 7 | 1 |

**Table 3*.*** Antimicrobial and clinical outcomes for the two groups. **Effective antibiotic 1** if the isolate was susceptible *in vitro* to that antibiotic, including combination with aminoglycosides. **Optimal antibiotic 2**, refers to the narrowest *in-vitro* effective antimicrobial.

|  |  |  |  |
| --- | --- | --- | --- |
| Antimicrobial outcomes | Rapid  N= 93 | Conventional  N= 98 | p-value |
| Total patients on empiric effective1 antibiotic before AST results | 87 (94%) | 93 (95%) | 1.00 |
| No of changes to effective antibiotic based on AST | 6 (6%) | 5 (5%) | 0.861 |
| Escalation (non-aminoglycoside) and discontinuation of aminoglycosides4 | 0 (0%) | 2 (40%) | 0.182 |
| Only escalation of non-aminoglycoside | 6 (100%) | 3 (60%) | 0.182 |
| Total patients in combination with aminoglycosides before AST results | 70 (76%) | 64 (65%) | 0.112 |
| No. of discontinuation of aminoglycosides based on AST | 40 (57%) | 24 (37%) | 0.038 |
| Only discontinuation of aminoglycosides | 19 (48%) | 15 (63%) | 0.305 |
| Escalation (non-aminoglycoside) and discontinuation of aminoglycosides | 21 (53%) | 9 (37%) | 0.305 |
| Total patients on optimal antibiotic 2 before AST results | 4 (4%) | 8 (8%) | 0.374 |
| No. of changes to optimal antibiotic based on AST results | 59 (93%) | 58 (88%) | 0.876 |
| Only escalation of non-aminoglycoside | 12 (20%) | 3 (5%) | 0.024 |
| Escalation (non-aminoglycoside) and discontinuation of aminoglycosides4 | 8 (9%) | 8 (14%) | 0.971 |
| Only discontinuation of aminoglycosides | 16 (27%) | 8 (14%) | 0.108 |
| De-escalation (non-aminoglycoside) | 23 (39%) | 39 (67%) | 0.003 |
| Length of stay (days); median (range) | 11 (0-47) | 10.5 (0-71) | 0.912 |
| Mortality at 28 days | 7 (8%) | 13 (13%) | 0.275 |

**Table 4*.*** Time-to-results and antibiotic outcomes for each group. Time from blood culture collection to antibiotic change, initiation or discontinuation. Median (range) in hours. Time to de-escalation refers to any class of non-aminoglycoside antibiotic. Abbreviations: AST: Antimicrobial Susceptibility Testing.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Time-to-antibiotic (hours) Median (range) | Rapid | | Conventional | | p-value |
|  | N, 93 | | N, 98 | |  |
| Time to positive blood culture | 19.15 | (2-106) | 21.8 | (2-110) | 0.372 |
|  | N, 93 | | N, 98 | |  |
| Time to AST communication of results | 33.1 | (17-123) | 55.2 | (33-145) | <0.001 |
|  | N, 93 | | N, 98 | |  |
| Time to first effective antimicrobial | 3 | (0-97) | 4 | (0-120) | 0.490 |
|  | N, 6 | | N, 5 | |  |
| Time to effective antimicrobial based on AST results | 39.5 | (32-97) | 57 | (49-83) | 0.036 |
|  | N, 93 | | N, 98 | |  |
| Time to effective non-aminoglycoside antibiotic | 14.52 | (0-145) | 20 | (0-142) | 0.827 |
|  | N, 33 | | N, 24 | |  |
| Time to effective non-aminoglycoside based on AST | 42.42 | (21-145) | 71.87 | (46-142) | 0.002 |
|  | N, 34 | | N, 15 | |  |
| Time to escalation (non-aminoglycoside) based on AST | 41.69 | (21-103) | 66.90 | (46-121) | 0.010 |
|  | N, 63 | | N, 66 | |  |
| Time to Optimal antimicrobial | 43 | (3-339) | 66.5 | (0-872) | 0.062 |
|  | N, 59 | | N, 58 | |  |
| Time to Optimal antimicrobial based on AST results | 50 | (10-339) | 69.5 | (20-872) | 0.034 |
|  | N, 70 | | N, 64 | |  |
| Time to stop aminoglycosides | 31.53 | (0-795) | 53.68 | (4-216) | 0.017 |
|  | N, 40 | | N, 24 | |  |
| Time to stop aminoglycosides based on AST results | 48.83 | (0-795) | 72 | (50-216) | 0.005 |
|  | N, 23 |  | N, 39 |  |  |
| Time to de-escalation (non-aminoglycosides) | 125.2 | (24-632) | 97.8 | (8-501) | 0.071 |
|  | N, 93 | | N, 98 | |  |
| Time to stop all antibiotics | 370.2 | (31-1126) | 335.6 | (50-1834) | 0.219 |

**Table 5.** Factors influencing discontinuation of aminoglycosides at 48 hours and reception of one dose of aminoglycosides. Results of the multinomial logistic regression. The results are expressed as adjusted odds ratio (OR) (95% confidence interval (CI)) for each of the variables included in the model. Only p-values below 0.3 in the univariate analysis were considered for the multivariate analysis.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Discontinuation of aminoglycosides at 48 hours versus >48 hours | | | | |
|  | **Univariate analysis** | | **Multivariate analysis** | |
| Variables | OR (95% CI) | P-value | OR (95% CI) | P-value |
| Above 65 yo (versus below 65 yo)  Medical specialty (versus surgical)  Other sources (versus urinary)  Penicillin allergy (versus no allergy)  Source control (versus no control)  Rapid AST (versus conventional) | 1.50 (0.73 – 3.05)  2.14 (0.97- 4.71)  **2.41 (1.19 – 4.87)**  0.52 (0.17 – 1.56)  1.68 (0.84 – 3.35)  **2.63 (1.30 – 5.32)** | 0.264  0.058  **0.014**  0.249  0.143  **0.007** | 1.40 (0.64- 3.07)  1.79 (0.77- 4.16)  **2.17 (1.02- 4.63)**  0.55 (0.16- 1.91)  1.59 (0.76- 3.33)  **2.30 (1.10- 4.80)** | 0.406  0.173  **0.045**  0.348  0.218  **0.027** |
| One dose of aminoglycosides versus two or more doses | | | | |
|  | **Univariate analysis** | | **Multivariate analysis** | |
| Variables | OR (95% CI) | P-value | OR (95% CI) | P-value |
| Other sources (versus urinary)  E.coli (versus other gram-negative)  Resistance 1 antibiotic group (versus >1)  Penicillin allergy (versus no allergy)  Rapid AST (versus conventional) | **2.71 (1.33-5.52)**  0.56 (0.27- 1.16)  2.17 (8.84-5.59)  **0.21 (0.05- 0.98)**  **3.77 (1.81- 7.89)** | **0.006**  0.119  0.108  **0.047**  **<0.001** | **2.53 (1.17-5.49)**  0.78 (0.35-1.76)  1.71 (0.61-4.78)  0.22 (0.04-1.11)  **3.20 (1.47- 6.98)** | **0.019**  0.546  0.303  0.066  **0.003** |

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**Figure 1.** Flowchart showing laboratory workflow and communication of results followed along the clinical service evaluation. Once a blood culture bottle flagged positive, Gram-stain was performed to select only the Gram-negative blood culture isolates. A blood sample was loaded directly from the blood culture bottle onto the rapid system (Alfred 60AST). In parallel, a 4hours agar plate was inoculated to enable identification of the Gram-negative isolate by MALDI-TOF. The mean total time from positive blood culture to identification and rapid susceptibility results was 6 hours (approx.). The BD Phoenix™ automated susceptibility testing system (BD Diagnostics, Sparks, MD) (software version 5.02H/4.11B) was used as the routine laboratory method, as conventional patient care. Conventional susceptibility testing was loaded from the 4h plate isolate and the mean total time from positive blood culture to conventional susceptibility results was 20 hours. Results were communicated to the medical team as soon as they were available, between 12pm and 17pm.

**Figure 2*.*** Flowchart of study population. A total of 211 episodes of Gram-negative bacteraemia during the study period. Nine episodes were excluded, of which three were polymicrobial samples, three anaerobes, two recurrent bacteraemia episodes and one clinically non-significant isolate (*Prevotella salivae*). 202 episodes of Gram-negative bacteraemia were included in the final service evaluation. A total of 93 patients with Gram-negative bacteraemia received a rapid susceptibility testing and 98 patients had a conventional susceptibility testing done.



Rapid (n, 48) median (range): 24 h (0- 48).

Conventional (n, 29): 34.08 h (4-48); p= 0.015.

Rapid (n, 29) median (range) = 39.53 (10 – 72). Conventional (n, 15) median (range)= 64.53 (21- 72); p < 0.001.



Rapid (n, 38) median (range): 33.5 (10-68).

Conventional (n, 32) median (range): 55.5 (20-72); p <0.001.

**Figure 3*.*** Kaplan-Meier curves for (A) time to stop aminoglycosides at 48 hours; (B) time to stop aminoglycosides at 72 hours; (C) time to start optimal antibiotic at 72 hours**.** (D) time to escalate non-aminoglycoside antibiotic at 72 hours. Times to antimicrobial modification are are based on AST results.Rapid group (blue line), Standard group (red line).

B) 41.69

A) 41.69



D)

C)

Rapid (n, 29) median (range): 40.35 (21-66). Conventional (n, 10) median (range): 52.93 (46-72); p< 0.001