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Modification of the rapid antimicrobial susceptibility testing from blood culture protocol for a resource-limited setting

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Background: Sepsis is a medical emergency and rapid antimicrobial susceptibility testing (RAST) is essential for patient management. However, existing RAST protocols may be unsuitable for resource-limited settings due to the need for rapid species identification, which may require specialized equipment or expensive reagents.

Aims: To minimize the requirement of the RAST protocol while maintaining its efficacy, focusing on *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii* and *Staphylococcus aureus*.

Methods: Positive blood cultures suspected of having these pathogens underwent RAST with three main modifications: delayed species identification, implementation of pan-species breakpoints and the change in the quality control process. Twelve antimicrobials were tested for Gram-negative bacilli, and three for *S. aureus*. Species identification was performed by both the MALDI-TOF MS and the rapid phenotypic tests at the final RAST time point. The categorical agreement was evaluated against the standard AST method and the RAST protocol.

Results: Among 398 samples, gentamicin, ampicillin, meropenem and trimethoprim-sulfamethoxazole met the accuracy criteria for Gram-negative bacilli. Ceftriaxone, imipenem and ciprofloxacin had slightly reduced agreement (80%–90%) due to a high false resistance. The remaining antimicrobials either had a low agreement or high false susceptibility. Only gentamicin passed the agreement criteria for *S. aureus*. The use of pan-species breakpoints resulted in several failed results without improvement in the concordance. The quality control process with and without sheep blood yielded comparable results.

Conclusion: RAST reduced the time-to-result for key antimicrobial agents for at least 24 h while requiring minimal workflow disruption, enabling early adjustment of antimicrobial treatment.

Introduction

Blood culture is a standard laboratory investigation for the detection of bacterial pathogens in patients with sepsis,[1] and the subsequent antimicrobial susceptibility testing (AST) either by the conventional phenotypic or the newly-developed genotypic methods[2] is essential for the proper management of the patients. As blood is often not the specimen from the site of infection but rather receives bacterial spillage from other sites, the number of bacterial cells in the blood is generally less than that obtained directly from the infection sites. It thus requires an initial culture in the blood culture broth.[3] Owing to this, the time-to-result of the blood culture and AST is longer than that of the culture from other specimens. To address this, several methods have been developed, including direct species identification from the positive blood culture broth,[4] the genotypic panel for both the identification and resistance detection,[2] or even methods that do not rely on blood culture.[5]

Despite the availability of genotypic resistance detection panels,[2] phenotypic AST remains the gold standard.[3] In recent years, EUCAST and, subsequently, CLSI developed a rapid AST (RAST) protocol directly from positive blood culture for selected pathogenic bacteria.[6–8] This greatly reduces the time to phenotypic AST results. However, the benefit of this protocol relies on the rapid identification of the causative pathogen, which may add cost to the laboratory process. For instance, direct identification by MALDI-TOF MS from positive blood culture requires an additional reagent on top of the already expensive process.[9]

This proves to be a burden for implementing this protocol, especially in resource-limited settings. Since the RAST protocol requires the reading at 16–20 h after incubation,[6–8] species identification may potentially be delayed until then. This would greatly reduce the testing cost without further delaying the test results.

Although performing MALDI-TOF on the bacterial colony at a 20-h time point is cheaper than the direct MALDI-TOF on the positive blood culture, it is still considered a resource-intensive process. Cheaper alternatives, such as the use of rapid phenotypic tests, can classify organisms into groups, but not species.[10] Still, it may be possible to define a set of multi-species breakpoints for each group of organisms, such as the breakpoints for Enterobacterales in the CLSI guideline,[7] further reducing the need for rapid species identification.

Another step that also requires additional resources is the quality control process, as either sterile defibrinated horse blood or sheep blood must be added to the blood culture bottle.[11] Given that the standard organisms (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) can grow in the blood culture broth in the absence of blood, it may be possible to perform the quality control process without the use of horse or sheep blood. Although this will have to be validated.[12]

This study aims to modify the RAST protocol to fit the needs in a resource-limited setting, using the EUCAST protocol as a blueprint,[6,8] supplemented by the CLSI protocol.[7] The protocol focuses on performing the RAST on all positive blood culture broths suspected of having the bacteria of interest while delaying the species identification process towards the final time point. We also evaluated the quality control process without the addition of sheep blood. We believe that these modifications of the RAST protocol remains efficient, at least for a subset of antimicrobial agents, while requiring minimal changes from the pre-existing standard laboratory protocols.

Materials and methods

Study site and settings

This study was conducted at the Bacteriology Laboratory, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. This laboratory receives clinical specimens from many clinical services; primarily Siriraj Hospital (public hospital, ~2100 inpatient beds) and Siriraj Piyamaharajkarun Hospital (private hospital, ~320 inpatient beds). Based on the 2024 statistics, this laboratory received an average of 11 500 samples for bacterial culture per month, 4930 (42.9%) being blood culture with ~11.1% (quartile range 10.7%–11.5%) positivity. Two automated blood culture systems were used in this laboratory; the BD BACTEC™ (Becton Dickinson, USA) and the BacT/ALERT (bioMérieux, France) system. Starting from November 2023 to April 2024, the consecutive 500 positive blood culture broths meeting the inclusion criteria (see below) were included in the study.

Proposed modified RAST protocol

Figure 1 summarizes different identification and susceptibility testing protocols following initial positive blood culture broth;[2,4,7,8,13] including methods that provide same-day results,[2,4] a fully automated method that provides results within one day,[13] the standard RAST protocol[8] and two variations of the proposed modified RAST protocol (this study, star), compared with the standard AST protocol.[7] The modified RAST should provide species identification and preliminary AST results for essential antimicrobials within one day of positive blood culture broth compared with two days by the standard AST protocol (50% time reduction). For this study, MALDI-TOF was used for species identification. Indeed, it is considered resource-intensive, but it is relatively cheaper than other methods[2,4,13] and can also be used for different purposes. A MALDI-TOF-independent variation, relying mainly on rapid biochemical tests, was also proposed.

Standard blood culture protocol

All blood culture broths were inoculated using the automated system according to the relevant protocol. Gram staining would be performed on the positive broth, and the preliminary results were reported. The broth would then be inoculated onto sheep blood agar and McConkey agar and incubated overnight (35°C, atmospheric condition). Species identification was performed on each unique colony on the agar plate using the MALDI-TOF MS (Becton Dickinson, USA). One of the three standard AST methods (disc diffusion, VITEK® 2 [bioMérieux, France] or Sensititre™ [Thermo Fisher Scientific, USA]) was also performed on each identified bacterium. The CLSI guidelines using zone diameter and MIC for AST interpretation were followed.[7]

RAST protocol

RAST protocols were described by both EUCAST and CLSI.[6–8] The differences between the two recommendations are summarized in Table 1. Briefly, CLSI recommendation is more limited than that of EUCAST in terms of the number of species and antimicrobial agents that can be tested. CLSI also recommend reading at either 8–10 h or 16–18 h time point, with an intermediate result if the inhibition zone falls between the susceptible and resistant breakpoints.[7] EUCAST recommends sequential reading starting at 4 h time point. If the inhibition zone is unclear or falls between the susceptible and resistant breakpoints, the result will not be interpreted but will be deferred to the reading at subsequent time points. There is no intermediate reading in the EUCAST recommendation.[6,8]

In parallel with the standard protocol stated above, the study protocol was also conducted in selected positive broths suspected of having the following organisms; *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Staphylococcus aureus*. All positive broths with the following Gram stain results were included: Gram-negative regular bacilli, Gram-negative coccobacilli and Gram-positive cocci in large clusters. RAST was performed on each specimen according to the EUCAST guideline.[8] Briefly, 100–150 μL of positive blood culture broth was inoculated on the Müller-Hinton plate and spread evenly. Antibiotic discs (Table S1, available as Supplementary data at *JAC-AMR* Online) were then placed onto the plates and the plates were then incubated. Each plate was taken out of the incubator at 4, 6, 8 and 16–20 h and the visible inhibition zones were recorded.

The 20-h time point would coincide with the species identification step in the standard protocol above (MALDI-TOF-MS). The positive broths with the final identification different from the bacteria of interest and positive broths with mixed growth of more than one organism were excluded from the study.

After species identification, the results were interpreted according to the EUCAST guidelines.[8] Briefly, if the inhibition zone was visible at 4 h and the zone diameter fell either in the susceptible or resistant range, the interpretation was recorded accordingly. If the inhibition zone was not visible or if the zone diameter fell between the susceptible and resistant range—the area of technical uncertainty (ATU), the interpretation was not recorded, and the plate would be re-read at the subsequent time points (up to four different time points). If the results could not be interpreted at the final time point (16–20 h), the interpretation was recorded as fail.

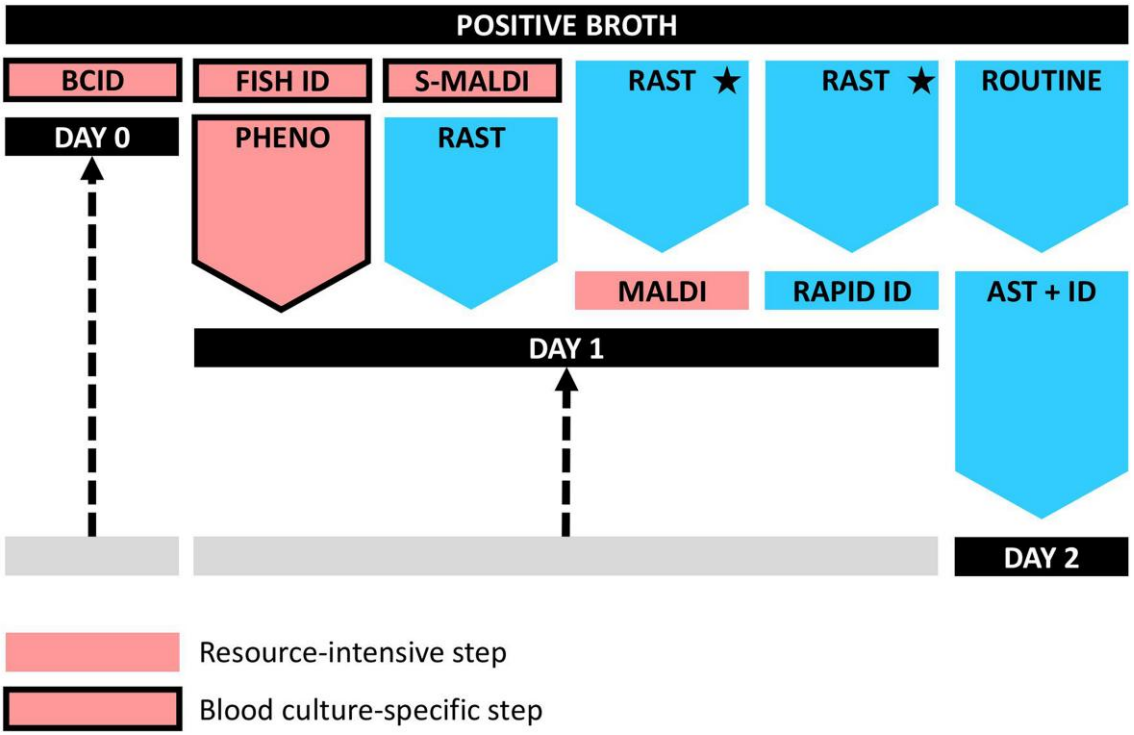

Figure 1. Comparison of species identification and AST protocols for positive blood culture. From left to right: [1] a genotypic method with same-day results, [2] an automated fluorescent *in situ* hybridisation with rapid phenotypic AST (Accelerate Pheno™; Accelerate Diagnostics, AZ), [3] the standard RAST protocol with direct MALDI-TOF from the positive broth (Sepsityper™; Bruker, MA), [4] the modified RAST protocols with the use of the MALDI-TOF system from the colonies on the agar plates (standard MALDI-TOF), [5] the modified RAST protocol with rapid biochemical test (oxidase) and pan-species breakpoints for oxidase-negative organisms, [6] the standard AST protocol. Resource-intensive steps refer to steps that require reagents/equipment that may not be present in some microbiology laboratories. Blood culture-specific steps refer to steps that require specific reagents/equipment for this protocol.

Acceptable criteria for routine implementation

The RAST results were then compared with the standard AST results using the categorical agreement according to Cumitech 31a.[12] The acceptable criteria for routine implementation of RAST are as follows: [1] the categorical agreement is at least 90.0%, [2] the very major errors (VMEs; false susceptible results) are not >1.5% and [3] the combined major errors (MEs; false resistant results) and VMEs are not >3.0%. Routine implementation of RAST as a sole testing method would be considered for antimicrobial agents that pass all three criteria. Confirmation by an alternative method would be needed for those not meeting one or more of the criteria.

Modification of the interpretation for RAST protocol

Upon initial interpretation and analyses, a high occurrence of MEs was noted (see below and Table S2). This was mainly due to the difficulty in reading and interpreting the inhibition zones at the 4 h time point. Thus, the interpretation protocol was adjusted, and the 4 h time point was omitted. This greatly improved the ME occurrence while minimally affecting other results (see results below).

Pan-species AST analysis from the RAST protocol

Since the MALDI-TOF system is considered a resource-intensive method, a variation of the modified RAST protocol was proposed. This version relies on the oxidase test to differentiate *P. aeruginosa* from other Gram-negative bacillary species (*E. coli*, *K. pneumoniae* and *A. baumannii*). The result interpretation for *P. aeruginosa* was similar to the protocol above. For the other three species, a set of pan-species breakpoints was used. These breakpoints utilizes the strictest zone of all three organisms according to the EUCAST recommendation (the largest susceptible zone and the smallest resistant zone among all three species) to minimize false interpretation in exchange for a wider ATU. The efficacy of the pan-species breakpoints was compared with the species-specific breakpoints.

Implementation of RAST protocol in routine services

After the initial study period and the modification of the RAST protocol, it was implemented in the clinical laboratory services at the study site, focusing on blood culture broths positive for Gram-negative bacilli. All specimens were processed as described above and the results were recorded in a spreadsheet. Potential result errors may need further confirmation. Quality control was performed 3 times a week on alternate days using *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 each with 5 mL sheep blood as recommended by EUCAST,[8,11] as well as without sheep blood. After the species identification and the initial RAST protocol, the medical technologist would report the initial results and then perform standard AST protocols for antimicrobials not included in the RAST panel and those that need repeating. The final report would be available on the subsequent day. During this period, the number of broths excluded and the number of antimicrobials needed to be repeated were recorded.

Statistical analyses

All data were interpreted and visualized using Tableau 2022.1 (Salesforce, San Francisco, CA). All statistical analyses in this study were performed

Table 1. Comparison between CLSI and EUCAST recommendation for RAST

Parameter	CLSI	EUCAST
Number of tested antimicrobials	More limited	—
Organisms (GN)	Enterobacterales	*Escherichia coli*
	Pseudomonas aeruginosa	*Klebsiella pneumoniae*
	Acinetobacter baumannii	*Pseudomonas aeruginosa*
		Acinetobacter baumannii
Organisms (GP)	—	*Staphylococcus aureus*
		Streptococcus pneumoniae
		Enterococcus faecalis
		Enterococcus faecium
Reading time point	—	4 h
	—	6 h
	8–10 h	8 h
	16–18 h	16–20 h
Reading method	At either time point	First time point with results
Quality control	Daily OR	Daily OR
	Weekly	At least four times a week
Updates	Annually	More frequent than annually
Accessibility	Subscription-based	Open access
Different antimicrobials	Ceftriaxone (30 µg)	Cefotaxime (5 µg)
	Ceftazidime (30 µg)	Ceftazidime (10 µg)
	Piperacillin/tazobactam (100/10 µg)	Piperacillin/tazobactam (30/6 µg)
Intermediate results	Report as intermediate	Read the next time point
		At the last time point, report ATU

using online tools available at Social Science Statistics (available at https://www.socscistatistics.com/) and Statistics Kingdom (available at https://www.statskingdom.com/). A *P*-value of <0.05 was considered to be significant.

Ethics approval

This study was exempted by the Siriraj Institutional Review Board (SIRB); protocol number 794/2566(Exempt).

Results

Study samples

Initially, a total of 500 positive broths were included in this study. Of these, 102 (20.4%) were excluded because the final identification did not match the bacteria of interest, and 36 (7.2%) were excluded due to the presence of more than one organism. The proportion of excluded broths was significantly higher in broths with Gram-positive cocci (39.5%) versus Gram-negative bacilli (25.5%; $P=0.018$). The number of isolates of each bacterium of interest was as follows: *E. coli* (160; 44.2%), *K. pneumoniae* (74; 20.4%), *A. baumannii* (44; 12.2%), *P. aeruginosa* (38; 10.5%) and *S. aureus* (46; 12.7%). The resistance prevalence based on the standard AST methods is summarized in Table 2.

Correlation between RAST and standard AST protocols

For the organism-antimicrobial pairs with standard breakpoints,[7,8] the overall correlation between RAST and standard AST protocols is summarized in Table 3 and the detailed correlation for each species is summarized in Table S2. From 11 antimicrobials with breakpoints for Gram-negative bacteria, four (36.4%; gentamicin, ampicillin, meropenem and trimethoprim-sulfamethoxazole) passed all the acceptable criteria, three (27.3%; ceftriaxone, imipenem and ciprofloxacin) did not pass the criteria due to high ME occurrence (false resistance), but the percent agreement was relatively high (>80.0%), another three (27.3%; amoxicillin-clavulanate, ceftazidime, cefepime) had decreased percent agreement (between 50.0%–80.0%, and one (9.1%; amikacin) had high VME occurrence (4.8% VME). From 3 antimicrobials with breakpoints for *S. aureus*, only gentamicin passed all the criteria. Cefoxitin had high ME rates but still had a relatively high percent agreement (91.3%), while clindamycin had poor overall results (52.6% agreement, 44.7% ME, 2.6% VME).

Despite the high ME rate, the agreement for *S. aureus* and cefoxitin was high. A closer look at the inhibition zones at different time points between strains with true resistance (true MRSA; $n=5$) and false resistance (MSSA; $n=3$) revealed that all MRSA strains had inhibition zones less than the resistance cut-off at every time point, while the MSSA strains only had one time point that fell into the resistance range.

Comparison between pan-species and species-specific breakpoints

To eliminate the need for the MALDI-TOF system, a variation of the RAST protocol relying on the oxidase test was used. In this protocol, *E. coli*, *K. pneumoniae* and *A. baumannii* were grouped as oxidase-negative Gram-negative bacilli and the AST results

Table 2. Percent resistance to selected antimicrobials by standard method

Antimicrobials	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	*Staphylococcus aureus*
Individual agent					
Amikacin	2.5	12.2	—	75.0	—
Gentamicin	30.0	20.3	—	79.5	4.3
Ampicillin	85.0	100.0	—	—	—
Amoxicillin-clavulanate	16.9	25.7	—	—	—
Piperacillin-tazobactam	8.1	29.7	21.1	75.0	—
Ceftriaxone	45.6	37.8	—	79.5	—
Ceftazidime	22.5	27.0	31.6	75.0	—
Cefepime	35.6	27.0	28.9	75.0	—
Imipenem	1.3	12.2	28.9	75.0	—
Meropenem	1.3	13.5	34.2	75.0	—
Ciprofloxacin	65.6	48.6	26.3	81.8	—
Trimethoprim-sulfamethoxazole	60.0	31.1	—	75.0	—
Cefoxitin	—	—	—	—	10.9
Clindamycin	—	—	—	—	8.7
Erythromycin	—	—	—	—	15.2
Multiple agents					
Multi drug-resistance[a]	60.6	43.2	26.3	75.0	—

[a]Defined as isolates that were resistant to three or more antimicrobial classes.

were interpreted using the pan-species breakpoints, which are stricter than the species-specific breakpoints. Table 4 compares the efficacy of these two sets of breakpoints. Overall, the pan-species breakpoints were associated with significantly more failed read counts, with almost no significant difference in concordance with the standard AST results.

Implementation of RAST protocol in routine services

After the initial study period, RAST was implemented in the routine services. As the exclusion proportion for Gram-positive cocci was high (39.5%) and the number of antimicrobial agents passing the acceptable criteria was low, RAST was only implemented for positive broths with Gram-negative bacilli, and the list of antimicrobials was chosen based on the initial results in Table S2 and the details are summarized in Table S3. A total of 55 blood culture broths underwent the RAST protocol during the first 90 days, and 18 were excluded (32.7%, not significantly different from previously expected, $P=0.250$); 14 had other species and 4 had more than one organism, leaving 37 specimens for the analysis (*E. coli* 48.6%, *K. pneumoniae* 24.3%, *A. baumannii* 13.5%, *P. aeruginosa* 13.5%). From 193 organism-antimicrobial data points, 8 (4.1%) were in the ATU (ceftriaxone 5 data points, ciprofloxacin 2 data points, imipenem 1 data point) and were excluded from the analysis. From the remaining 185 data points, 7 (3.8%) had minor errors (ciprofloxacin 5 data points, gentamicin 1 data point, imipenem 1 data point) and 2 (1.1%) had major errors (gentamicin 1 data point, ceftriaxone 1 data point).

Modification of the quality control process

As the two control strains, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, do not require sheep blood for the growth in the blood culture broth, this study also evaluated the quality control process without the supplementation of sheep blood to the standard recommendation. The zone of inhibition for all antimicrobial agents except for ceftriaxone for *P. aeruginosa* had minimal difference between the two methods (average absolute difference 0.6 ± 0.3 mm for *E. coli* and 0.9 ± 0.4 mm for *P. aeruginosa*), without the change in the pass/fail final results.

Discussion

Rapid species identification and AST from positive blood culture can greatly help with patient care in the critical phase of sepsis management, especially in the choice of appropriate antimicrobial treatment.[1] In recent years, several new methods have been developed to bypass the need to subculture the positive blood culture broth.[2] While these methods greatly reduce the time to result in the investigation of sepsis, these methods usually come with high costs or complex protocols, resulting in a difficult uptake, especially in resource-limited settings. Among these methods, disc diffusion RAST protocols are widely validated,[7,8] relatively cheap and do not require additional equipment beyond the standard AST protocol.[6,7] However, the standard RAST protocol still requires rapid species identification, which can still be resource-intensive.[2,4] Given that the final time point of the RAST protocol is at 16–20 h,[7,8] allowing time for the bacterial growth on the initial subculture. The species identification can be moved to the end of the protocol, reducing the total cost of the process.

Since the species identification was at the end of the protocol, it was expected that some specimens would have to be retrospectively excluded. In this study, the proportion of the excluded specimens was 25.5% for Gram-negative bacilli and 39.5% for Gram-positive cocci. Given that there is a higher prevalence of

Table 3. Correlation between standard and RAST protocol

Antimicrobials	Agreement	mE	ME	VME	ME + VME
Amikacin	90.1	3.7	1.5	4.8	6.3
Gentamicin (overall)	98.1	0.0	0.6	1.2	1.9
Gentamicin (Gram-negative)	98.2	0.0	0.4	1.4	1.8
Gentamicin (*S. aureus*)	97.8	0.0	2.2	0.0	2.2
Ampicillin	98.7	0.0	1.3	0.0	1.3
Amoxicillin-clavulanate	55.1	9.8	35.0	0.0	35.0
Ceftriaxone	84.1	3.5	12.4	0.0	12.4
Ceftazidime	71.6	9.2	19.3	0.0	19.3
Cefepime	62.2	2.2	35.6	0.0	35.6
Imipenem	95.5	1.0	3.5	0.0	3.5
Meropenem	95.3	1.9	2.5	0.3	2.8
Ciprofloxacin	87.6	7.2	3.9	1.3	5.2
Trimethoprim-sulfamethoxazole	97.1	0.7	1.8	0.4	2.2
Cefoxitin	91.3	0.0	8.7	0.0	8.7
Clindamycin	52.6	0.0	44.7	2.6	47.4

mE, minor error (discrepancy between intermediate and susceptible/resistant results); ME, major error (false resistance); VME, very major error (false susceptible).

Table 4. Comparison between pan-species and species-specific breakpoints for oxidase-negative Gram-negative bacilli

Antimicrobials	Breakpoints	Read counts	*P*	Concordance	*P*
Amikacin	Pan-species	214 (77.0%)	<0.001	194 (90.7%)	0.462
(*n* = 278)	Species-specific	277 (99.6%)		245 (88.5%)	
Gentamicin	Pan-species	259 (93.2%)	<0.001	256 (98.8%)	1.000
(*n* = 278)	Species-specific	277 (99.6%)		273 (98.6%)	
Ceftriaxone	Pan-species	151 (54.3%)	<0.001	143 (94.7%)	0.002
(*n* = 278)	Species-specific	226 (81.3%)		190 (84.1%)	
Ceftazidime	Pan-species	8 (2.9%)	<0.001	8 (100.0%)	0.057
(*n* = 275)	Species-specific	183 (66.5%)		122 (66.7%)	
Imipenem	Pan-species	160 (57.6%)	<0.001	159 (99.4%)	0.062
(*n* = 278)	Species-specific	276 (99.3%)		266 (96.4%)	
Meropenem	Pan-species	261 (93.9%)	<0.001	254 (97.3%)	0.477
(*n* = 278)	Species-specific	278 (100.0%)		267 (96.0%)	
Ciprofloxacin	Pan-species	245 (88.1%)	<0.001	221 (90.2%)	0.571
(*n* = 278)	Species-specific	272 (97.8%)		241 (88.6%)	
Trimethoprim-sulfamethoxazole	Pan-species	268 (96.8%)	0.004	261 (97.4%)	0.474
(*n* = 277)	Species-specific	277 (100.0%)		266 (96.0%)	

Gram-negative sepsis, a higher prevalence of antimicrobial resistance, a larger pool of antimicrobial agents to be tested, as well as a lower proportion of excluded specimens for Gram-negative bacilli, it is reasonable to implement the modified RAST protocol on this bacterial population.

For antimicrobial agents where standard breakpoints were available,[7,8] good correlations were observed in most cases. Although a few agents (e.g. ceftriaxone, imipenem and ciprofloxacin) had relatively high false resistance (major error) and did not pass the acceptable criteria,[12] We believe that the modified RAST protocol can still be implemented in these agents to expand the coverage of antimicrobial classes, given that the resistant results be confirmed by another standard method.

This study primarily used the EUCAST guideline because of the wider range of organisms and antimicrobial agents that can be tested.[8] Although breakpoints from the CLSI guideline were supplemented where there are discrepancies between the two guidelines (Table 1).[7] The EUCAST guideline utilizes the ATU range where the results cannot be interpreted, instead of the 'intermediate' results.[8] Thus, the strains with intermediate results by the standard protocol would either provide minor discordance or no result at all. This resulted in the high mE rate for some antimicrobial agents, one of which (ciprofloxacin) would have passed the agreement criterion of 90% if the intermediate strains were disregarded. To alleviate this, careful attention should be paid to strains with multiple ATU reads or strains with inhibition zones

around the cut-off, and a confirmation method may be needed to reduce the mE rate.

The study site is the reference laboratory in a medical school in Thailand and has access to the MALDI-TOF system. However, this is not the case for smaller laboratories in the countries, as well as most of the laboratories in resource-limited settings. Thus, a variation of the protocol relying on the oxidase test was also evaluated to further eliminate the need for the MALDI-TOF identification. Based on this rapid phenotypic test, *E. coli*, *K. pneumoniae* and *A. baumannii* would be grouped as oxidase-negative Gram-negative bacilli. The pan-species breakpoints were stricter than the species-specific ones to avoid categorical errors. As a result, there was a significant increase in the number of failed read counts, which would require repeats by the standard protocol. Despite the significantly lower number of successful read counts, the pan-species breakpoints were not associated with the increase in concordance with the standard AST results in most cases. This suggests that the inhibition zones for many bacterial strains were very close to the breakpoints[7,8] and accurate species identification is needed. To improve upon this, a commercialized rapid phenotypic assay [such as the RapID™ ONE System (Thermo Scientific, MA)], or additional spot tests (such as indole or pyrrolidonyl arylamidase test) may be used instead of the MALDI-TOF system.

This study also assessed whether the quality control process could be performed without the use of sheep blood as recommended by the EUCAST guideline,[11] to further reduce the resources. The omission of sheep blood in the quality control process resulted in a very minimal change in the size of the inhibition zone and no change in the final interpretation with only one exception: ceftriaxone for *P. aeruginosa*. Since there is no interpretation breakpoint for *P. aeruginosa* against ceftriaxone[7,8] and the results are not reported, this should not affect the overall performance of the modified RAST protocol.

A brief implementation of the limited RAST protocol was proven to be successful, with positive feedback from both clinicians and laboratory technicians. The major error rates were within the acceptable range and were all detected and corrected using the standard AST methods, resulting in minimal impact on the patients. Given the high prevalence of Gram-negative bacteraemia in Thailand and the high prevalence of antimicrobial resistance among the significant organisms,[14] the resource-adjusted RAST protocol is likely to be useful for appropriate antimicrobial management during the acute phase of sepsis management.[1]

Even though the results for *S. aureus* were largely unsatisfactory, it may be useful in some instances. For example, the cefoxitin screening using RAST had a very high concordance with the standard interpretation and would have a 100% concordance with a stricter interpretation (i.e. the strain must be resistant at all time points to be considered MRSA). This would allow for the rapid detection of MRSA bacteraemia without the use of any molecular methods, potentially aiding the treatment adjustment and infection control.

There are several limitations to the implementation of the RAST protocol during the study period. First, the time points for plate reading and interpretation span at least 16 h,[6–8] extending into the night shifts with more limited manpower compared with the day shifts. Thus, the RAST protocol will likely increase the technician's workload, especially when there are multiple positive broths in one day. Second, the interpretation of the RAST protocol is complicated, involving several interpretation tables.[8] This would also increase the workload on the technicians and may result in more human error. The use of the spreadsheet with incorporated breakpoints greatly alleviates this. Finally, there were a few strains with aberrant growth, which were responsible for a majority of major and very major error incidence in this study. The implementation of a backup AST protocol can greatly reduce this, although it will result in an increased workload, aggravating the first limitation of the RAST protocol.

In conclusion, the modified RAST protocol can be implemented in a resource-limited setting. By moving the species identification step towards the end of the RAST protocol, the cost of the process would be decreased with minimal effects on the efficacy. In this study, MALDI-TOF was used for species identification, although rapid phenotypic tests may be used to further reduce the cost. Furthermore, sheep blood may be omitted in the quality control process with very minimal impact on the overall performance. This modified protocol has been proven to be useful for the early management of Gram-negative sepsis, an important medical emergency.

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Transparency declarations

The authors declare no conflicts of interest.

Supplementary data

Tables S1 to S3 is available as Supplementary data at *JAC-AMR* Online.

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