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Original Article

Implementing EUCAST rapid antimicrobial susceptibility testing method for sepsis: lessons learned in a tertiary care center

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Abstract

Introduction: We prospectively evaluated EUCAST rapid antimicrobial susceptibility testing methodology for susceptibility testing directly from blood culture bottles in comparison to CLSI disk-diffusion method.

Methodology: During May-November 2019, positively flagged blood culture bottles showing Gram-negative micro-organisms were simultaneously processed by rapid antimicrobial susceptibility testing and CLSI methodology. Antibiotics tested were cefotaxime, ceftazidime, piperacillin-tazobactam, imipenem, meropenem, gentamicin, tobramycin and trimethoprim-sulphamethoxazole.

Results: Overall, 80 isolates identified as *Escherichia coli* (n = 24, 30%), *Klebsiella pneumoniae* (n = 15, 18.7%), *Pseudomonas aeruginosa* (n = 16, 20%) and *Acinetobacter baumannii* (n = 25, 31.2%) were included. Categorical agreements of rapid antimicrobial susceptibility testing at 4-, 6- and 8-hour reading times were 88.1% (304/345), 90.8% (425/468) and 92.3% (467/506), respectively. Major Error rates were 14% (21/150), 4.9% (10/206) and 4/236 (1.7%), whereas Very Major Error rates were 1.1% (2/177), 1.3% (3/232) and 3.3% (8/243), respectively. Results categorized as "Area of Technical Uncertainty" were significantly lower at 8-hour {10.2% (39/384) *vs* 5.2% (28/534), 4- *vs* 8-hour, $p = 0.003$, Fischer's exact test}.

Conclusions: Except for a slightly higher Very major error rate, rapid antimicrobial susceptibility testing at 8-hour is equivalent to Disk-diffusion method (CLSI-M100) using CLSI-M52 criteria for equivalence: (Categorical agreement ≥ 90%, Very major error ≤ 1.5% and Major error ≤ 3%). Poor Categorical agreements at all reading times were noted for piperacillin-tazobactam, ciprofloxacin and *E. coli*. Performance of rapid antimicrobial susceptibility testing methodology in resource limited settings brings unique challenge of identifying micro-organisms within 8 hours. We suggest reading and reporting of results at a single time point using rapid antimicrobial susceptibility testing method i.e. at 8-hour.

Key words: RAST; EUCAST; sepsis; antimicrobial susceptibility testing; antimicrobial stewardship.

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Introduction

Antimicrobial resistance (AMR) has been declared as a "Global Health Emergency" by United Nations [1]. By 2050, multi-drug resistant micro-organisms (MDROs) are expected to be the leading cause of mortality worldwide, whose major burden will be shouldered by developing countries [2]. Indiscriminate use of antibiotics coupled with poor infection control practices is the major driving force for development and spread of MDROs in developing countries [3].

Sepsis is one of the leading causes of mortality causing ≥ 24 million sepsis-related deaths annually [4]. Mortality rate in sepsis increases parallelly with disease severity, reaching to 40–80% in patients with septic shock [5]. Early institution of appropriate antimicrobial therapy improves patient outcomes in sepsis, as there is 9% increase in odds of mortality with each hour delay [6]. With currently available microbiological diagnostic tools, turnaround time (TAT) of a positive blood culture report with antimicrobial susceptibility testing (AST) results is about 72-96 hours. Consequently, broad-spectrum antibiotics are used empirically with impunity leading to AMR. Hence, there is a dire need to provide AST results in clinically meaningful timeframes to improve patient outcomes, reduce broad-spectrum antimicrobial use and limit AMR.

It is commendable that both Clinical and Laboratory Standards Institute (CLSI) and European

Committee on Antimicrobial Susceptibility Testing (EUCAST) have been working on establishing performance criteria for direct disk diffusion from positively flagged blood culture (BC) bottles [7-9]. In 2018, EUCAST came up with rapid antimicrobial susceptibility testing (RAST) methodology from positive blood culture bottles [8], wherein AST results can be interpreted as earliest in 4 hrs. We conducted this study with the aim of evaluating the performance of RAST methodology [9,10] in comparison with disk diffusion method by CLSI, which is routinely followed in our setting [11].

Methodology

Setting and Study Design

This study was conducted in an academic, 750 bedded, tertiary care hospital in India. The bacteriology laboratory is functional round the clock and is equipped with BacTAlert®-3D (bioMerieux, Marcy l'Etoile, France) continuous blood culture monitoring system. It was a prospective study carried out between May-November 2019 on positively flagged blood culture (BC) bottles showing Gram-negative bacilli/coccobacilli on Gram's staining. Only one isolate per patient was included in the study. Bottles showing ≥ 2 different micro-organisms on Gram's staining or culture were excluded.

Microbiological Methods

Positively flagged BC bottles showing Gram-negative micro-organisms were processed simultaneously as per RAST [10] as well as by routine methodology [11]. Briefly, 125 ± 25 µL of blood-broth inoculum was aspirated in 1 mL syringe and lawn culture was done on Mueller-Hinton agar (MHA, Himedia®, Mumbai, India) plates for RAST methodology. Following antibiotic discs (Himedia®, India) were placed on the MHA plates and incubated subsequently at 35 ± 2 °C, namely: ceftazidime (10 µg), cefotaxime (5 µg), piperacillin-tazobactam (30-6 µg), imipenem (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), tobramycin (10 µg) and trimethoprim-sulphamethoxazole (1.25-23.75 µg). Zone of inhibitions were read manually at 4-, 6- and 8-hour [10] within stipulated times.

Parallelly, inoculum from BC bottles was inoculated on chocolate agar, blood agar and MacConkey agar (Himedia®, Mumbai, India) plates. They were subsequently incubated for 18-20 hours for appearance of isolated colonies [11]. Identification of micro-organisms was performed by conventional biochemical tests [12] and susceptibility testing by Kirby-Bauer disk-diffusion method [11]. Results of antimicrobial susceptibility were interpreted as per CLSI-M100 [11]. Only those isolates which were identified as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex, were included in the study. Zone of inhibitions against *P. aeruginosa* were not interpreted at 4-hour as per RAST interpretive criteria. Quality control using *Escherichia coli* ATCC® 25922 was performed weekly as per RAST standard [9].

Comparison of RAST and CLSI-M100

Results obtained by RAST at different time points were compared to results obtained by routine methodology, considering latter as "gold standard". Results categorized as "Area of Technical Uncertainty" (ATU) in RAST methodology were excluded from analysis as RAST standard recommends against reporting such results [10]. Categorical agreement (CA) was considered when interpretive category for a particular drug:bug combination (e.g. Meropenem: *Escherichia coli*) was same by both methodologies, except for Ciprofloxacin: *Acinetobacter baumannii* complex, for which results categorized as "Susceptible, increased exposure" were considered equivalent to "Susceptible" in routine methodology. Results not in CA were further categorized as Major error (ME), Very Major Error (VME) and Minor Error (mE). A particular drug:bug AST result was categorized as ME or False resistant when it tested "Resistant" by RAST and "Susceptible" by routine methodology; VME or False susceptible when "Susceptible" by RAST and "Resistant" by routine methodology while all other discrepancies were categorized as mE [13]. Rates for CA, and mE were calculated by dividing the total number of results in each category (numerator) by total number of such drug-bug combinations tested (denominator) and multiplied by 100. Rates for ME of a particular drug:bug combination were calculated by dividing the number of false resistant results (numerator) by total number of susceptible results (denominator) for a particular drug:bug combination and multiplying by 100. Rates for VME were calculated by dividing the number of false susceptible results (numerator) by Total number of resistant results (denominator) and multiplying by 100.

Statistical Analysis

Data entry was done on Microsoft Excel and statistical analysis was done by applying Fisher's exact test for categorical variables, where appropriate. $p \leq 0.05$ was considered as "statistically significant".

Ethical Approval

This study was an *in-vitro* study and involved work only on microbiological isolates. Hence it was considered exempt from human ethical approval.

Results

We received 3406 blood culture (BC) bottles during the study period, of which 860 (25.2%) flagged positive. Two hundred five (205) of 860 (23.8%) positive bottles showed Gram-negatives on Gram's staining on which RAST was performed. Of them, 80 isolates which had monomicrobial growth of *E. coli* (n = 24, 30%), *K. pneumoniae* (n = 15, 18.7%), *P. aeruginosa* (n = 16, 20%) and *A. baumannii* (n = 25, 31.2%) were eligible for inclusion. The overall susceptibility profile of these isolates against tested antimicrobials, as per routine methodology is given in Figure 1.

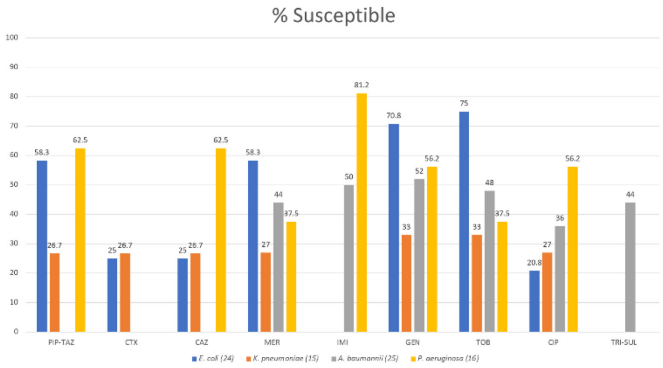
Of these 80 isolates, growth was visible in 68 (83.75%), 77 (96.2%) and 80 (100%) isolates at 4-, 6- and 8-hour, respectively. Of 12 isolates, in which growth was not visible at 4-hour, 6 (50%) were *P. aeruginosa*, 4 (33%) were *A. baumannii* complex and 1 (8.3%) each were of *E. coli* and *K. pneumoniae*. Of 3 isolates, in which growth was not visible at 6th hour, 2 (67%) were *P. aeruginosa* and 1 (33%) was *A. baumannii* complex. Median time interval between removal of positively flagged BC bottle from the system and performance of RAST methodology was 120 minutes (range: 10-300, Interquartile range: 60-180) in 70 isolates, for which data was available. The proportion of each category of results as per RAST, at different timepoints are shown in Table 1. These results were compared with the results obtained by routine methodology and are shown in Table 2. The results were also analysed in terms of variables such as antibiotics tested, micro-organisms and duration between positive signal by BC system and performance of RAST, as shown in Table 2.

Discussion

In this preliminary study, we evaluated RAST methodology by EUCAST against disk diffusion method by CLSI [11]. Our study showed that results of RAST methodology at 6- and 8-hour were equivalent to disk-diffusion method [11], except for slightly higher ME rate at 6-hour (4.9%) and VME rate at 8-hour (3.3%), when standard cutoffs for equivalence in susceptibility testing methods i.e. CA ≥ 90%, VME ≤ 1.5% and ME ≤ 3%), were applied [14]. At 4-hour, poor CA (88.1%) and unacceptably high ME rates (14%) were noted. We also noted slightly higher VME rate (3.3%) at 8-hour, majority of which were due to different zone diameters assigned for interpretive category determination in EUCAST and CLSI standards. Discrepancies in clinical breakpoints between EUCAST and CLSI are known and there are calls for harmonization between them [15].

Our results should be interpreted considering that 10.2% (39/384), 8.9% (46/514) and 5.2% (28/534) of AST results were categorized as "Area of Technical Uncertainty or ATU" at 4-, 6- and 8-hour, respectively and were excluded from the denominator while calculating Categorical agreement (CA). On sub-analysis of 39 AST results categorized as "ATU" at 4-

Figure 1. Antimicrobial susceptibility pattern of isolates included in the analysis* (As per Routine Methodology: Disk Diffusion, interpreted as per CLSI-M100-S29 [11]).



CLSI: Clinical and Laboratory Standards Institute, PIP-TAZ: piperacillin-tazobactam, CTX: cefotaxime, CAZ: ceftazidime, MER: meropenem, IMI: imipenem, GEN: gentamicin, TOB: tobramycin, CIP: ciprofloxacin, TRI-SUL: trimethoprim-sulphamethoxazole. *Data shown only for antibiotics interpretable for specific micro-organisms as per RAST interpretive standard (Version 1.1).

Table 1. Summary of Results of RAST methodology (version 1.1) and Routine Methodology [11].

RAST Methodology									Routine Methodology (Denominator: 534)		
4 Hour (Denominator: 384)			6 Hour (Denominator: 514)			8 Hour (Denominator: 534)					
S/SIE	ATU	R	S/SIE	ATU	R	S/SIE	ATU	R	S	I	R
142 (37%)	39 (10.2%)	203 (52.9%)	216 (42%)	46 (8.9%)	252 (49%)	261 (48.9%)	28 (5.2%)	245 (45.9%)	241 (45.1%)	42 (7.9%)	251 (47%)

RAST: Rapid Antimicrobial Susceptibility Testing; CLSI: Clinical Laboratory Standards Institute; S: Susceptible; SIE: Susceptible Increased Exposure; ATU: Area of Technical Uncertainty; R: Resistant; I: Intermediate.

hour, we noted that 67% (26/39) were interpreted as "Sensitive" at 8-hour whereas 7.7% (3/39) were interpreted as "Resistant". Corresponding results of "ATU" at 6-hour were 54% (25/46) and 4% (2/46), respectively implying that "ATU" results at earlier time points had more probability of getting recategorized as "Sensitive" rather than "Resistant", when read at later time points. Furthermore, 53% (15/28) of AST results, interpreted as "ATU" at 8-hour in RAST methodology, tested "Intermediate" in CLSI methodology. Corresponding results at 6- and 4- hour were 24% (11/46) and 18% (7/39). This shows that results under "ATU" category at later time points had more concordance with "Intermediate" category of CLSI. Furthermore, we also found a statistically significant reduction (10.2% vs 5.2%, 4- vs 8-hour, p=0.003, Fischer's exact test) in the proportion of results in "ATU" category at 8-hour. All these observations are expected as the zone diameters are dynamic in nature and are not fully established during early phase of disk diffusion testing thus showing false resistance. Overall, these results conclude that results of RAST methodology will give more confidence in reporting when readings are taken at later time points.

We compared results obtained at different timepoints to select the most suitable timepoint at which the results can be provided to clinicians. We noted that 15% (57/384) of drug:bug testing results had discordant interpretive categories between readings at 4-, 6- and 8-hour and 9% (45/514) between readings at 6- and 8-hour. This has practical implications as RAST standard doesn't provide a timepoint at which reports can be communicated to clinicians which will enable them to re-evaluate antibiotic therapy. Providing 2-hourly

Table 2. Comparison of RAST Methodology (EUCAST, version 1.1) with Routine Methodology [11] in terms of Categorical Agreement, Major Errors, Very Major Errors and Minor Errors at 4, 6- and 8-hour of incubation.

Variables (Denominator at 4, 6, and 8 hour of reading)	4 hour				6 hour				8 hour			
	CA n/d (%)	ME n/d (%)	VME n/d (%)	mE n/d (%)	CA n/d (%)	ME n/d (%)	VME n/d (%)	mE n/d (%)	CA n/d (%)	ME n/d (%)	VME n/d (%)	mE n/d (%)
Micro-organisms												
E. coli	117/138 (84.8%)	9/68 (13.2%)	1/59 (1.7%)	11/138 (8%)	135/154 (87.7%)	4/76 (5.3%)	-/63	15/154 (9.7%)	138/156 (88.5%)	2/79 (2.5%)	3/64 (4.7%)	13/156 (8.3%)
K. pneumoniae	89/94 (94.7%)	1/28 (3.6%)	1/63 (1.6%)	3/94 (3.2%)	95/99 (94.7%)	-/27	2/70 (2.9%)	2/99 (2%)	98/103 (95.1%)	-/30	2/70 (2.9%)	3/103 (2.9%)
A. baumannii	93/108 (86.1%)	11/54 (20.4%)	-/55	4/108 (3.7%)	124/132 (93.9%)	4/60 (6.7%)	-/68	4/132 (3%)	136/143 (95.1%)	2/66 (3%)	1/73 (1.4%)	4/143 (2.8%)
P. aeruginosa	Interpretive Criteria Not Available				71/83 (85.5%)	2/43 (4.7%)	1/31 (3.2%)	9/83 (10.8%)	95/104 (91.3%)	-/61	2/36 (5.6%)	7/104 (6.7%)
Antibiotics												
PIP-TAZ	26/30 (86.7%)	1/13 (7.7%)	-/14	3/30 (10%)	40/46 (87%)	1/23 (4.3%)	-/18	5/46 (10.9%)	44/49 (89.8%)	-/27	-/17	5/49 (10.2%)
CTX	32/35 (91.4%)	1/10 (10%)	-/23	2/35 (5.7%)	34/35 (97.1%)	-/9	-/25	1/35 (2.9%)	35/36 (97.2%)	-/10	-/25	1/36 (2.8%)
CAZ	30/34 (88.2%)	1/10 (10%)	-/21	3/34 (8.8%)	43/50 (86%)	-/16	1/28 (3.6%)	6/50 (12%)	47/52 (90.4%)	-/20	1/28 (3.6%)	4/52 (7.7%)
MER	49/52 (94.2%)	-/23	1/27 (3.7%)	2/52 (3.8%)	68/73 (93.2%)	-/32	1/37 (2.7%)	4/73 (5.5%)	72/78 (92.3%)	1/35 (2.9%)	2/40 (5%)	3/78 (3.8%)
CIP	40/49 (81.6%)	3/13 (23.1%)	1/31 (3.2%)	5/49 (10.2%)	58/67 (86.6%)	1/19 (5.3%)	-/40	8/67 (11.9%)	64/76 (84.2%)	1/26 (3.8%)	3/42 (7.1%)	8/76 (10.5%)
GEN	45/52 (86.5%)	7/30 (23.3%)	-/22	-/52	65/72 (90.3%)	4/41 (9.8%)	1/29 (3.4%)	2/72 (2.8%)	73/75 (97.3%)	1/44 (2.3%)	1/31 (3.2%)	-/75
TOB	50/54 (92.6%)	3/30 (10%)	-/22	1/54 (1.9%)	67/72 (93.1%)	2/38 (5.3%)	-/31	3/72 (4.2%)	73/78 (93.6%)	1/40 (2.5%)	-/34	4/78 (5.1%)
IMI	15/19 (78.9%)	3/10 (30%)	-/9	1/19 (5.3%)	31/32 (96.9%)	1/18 (5.6%)	-/14	-/32	38/39 (97.4%)	-/24	-/14	1/39 (2.6%)
TRI-SUL	17/20 (85%)	2/11 (18.2%)	-/8	1/20 (5%)	19/21 (90.5%)	1/10 (10%)	-/10	1/21 (4.8%)	21/23 (91.3%)	-/10	1/12 (8.3%)	1/23 (4.3%)
Duration Between Positive flagging of Blood culture bottle & Processing of RAST (Data shown for 70 isolates)												
Within 2 hours	154/180 (85.6%)	16/89 (18%)	-/81	10/180 (5.5%)	205/232 (88.4%)	9/116 (7.8%)	1/99 (1%)	17/232 (7.3%)	228/246 (92.7%)	2/132 (1.5%)	2/100 (2%)	14/246 (5.7%)
More than 2 hours	125/135 (92.6%)	4/40 (10%)	1/87 (1.1%)	5/135 (3.7%)	169/179 (94.4%)	1/64 (1.6%)	2/108 (1.9%)	7/179 (3.9%)	185/195 (94.9%)	2/77 (2.6%)	4/114 (3.5%)	5/195 (2.6%)
Overall												
RAST Results	304/345 (88.1%)	21/150 (14%)	2/177 (1.1%)	18/345 (5.2%)	425/468 (90.8%)	10/206 (4.9%)	3/232 (1.3%)	30/468 (6.4%)	467/506 (92.3%)	4/236 (1.7%)	8/243 (3.3%)	27/506 (5.3%)

RAST: Rapid Antimicrobial Susceptibility Testing; EUCAST: European Union Committee on Antimicrobial Susceptibility Testing; CLSI: Clinical and Laboratory Standards Institute; n: numerator; d: denominator; CA: Categorical Agreement; ME: Major Error; VME: Very Major Error; mE: Minor Error; PIP-TAZ: piperacillin-tazobactam; CTX: cefotaxime; CAZ: ceftazidime; MER: meropenem; CIP: ciprofloxacin; GEN: gentamicin; TOB: tobramycin.

reports will be labour intensive and fluctuating results between them will not help clinicians in deciding the most appropriate antibiotic therapy. Hence, we opine that the reports should be provided to clinicians at a single and most accurate time point. As per our study, the results were most concordant with CLSI methodology when plates were read at 8-hour.

To date, only two studies have evaluated RAST methodology by EUCAST on clinical samples in their settings [16,17]. In both studies, micro-organisms were identified using MALDI-TOF and AST results were compared with VITEK®-2 system using EUCAST breakpoints. Soo et al [17] evaluated RAST methodology (version 1.1) at 4-, 6- and 8-hour, whereas Jasuja et al [16] evaluated at 6-hr (version 1) only. Both studies found RAST to be equivalent to AST by VITEK®-2 system, the former [17] at all time points. In comparison, ours being a resource limited setting, micro-organisms were identified using battery of in-house prepared biochemicals and AST was done by Kirby-Bauer disk-diffusion method.

Of various antibiotics tested, poor CAs were seen for piperacillin-tazobactam and ciprofloxacin at all time points (Table 2). Similar findings were noted in previously conducted studies as well [16,17]. However, majority of discordant results in our study were categorized as minor errors which could be due to different zone diameters for deciding interpretive category between EUCAST and CLSI. The CAs of 3rd generation cephalosporins, carbapenems and aminoglycosides were within acceptable limits at 8-hour. Of various Gram-negatives reportable by RAST, poor CA was noted with *E. coli* only (Table 2). We also found that when duration between positive signal and processing of RAST was >2 hours, CAs were >90% at all timepoints, however when processing was done within ≤ 2 hours, acceptable results were obtained only at 8-hour (Table 2). Presently, EUCAST standard recommends RAST can be performed within 18 hours, if bottles are not removed from the instrument. In any AST method, bacterial inoculum is a critical variable which should be uniform, however, while doing susceptibility testing directly from a specimen, this is extremely difficult to control. Thus, further detailed studies are required to assess the effect of this variable on the accuracy of RAST methodology.

RAST methodology can reduce TAT of positive blood culture bottles considerably. Studies have shown benefit of 16-20 hours by using RAST methodology for AST [16,17]. It will improve patient outcomes by quickly escalating to appropriate antimicrobial therapy. Clinicians are often reluctant to de-escalate antibiotic regimen after 3-4 days of a successful broad-spectrum empirical antibiotic regimen [18]. As a result, this first line antimicrobial stewardship act has become more of an academic jargon. By providing an early window to clinicians for re-evaluating the broad-spectrum antimicrobial therapy, compliance to de-escalation of antibiotic therapy can be improved.

In our opinion, RAST methodology has great potential to reduce reporting time of microbiologically positive blood cultures and is ideally suited for resource limited settings, since it doesn't require new instrumentation, is less costly, can test multiple classes of antibiotics simultaneously and performance is relatively simple. The technique is ideally suited for Gram-negative micro-organisms, as their growth rate makes it easier to read susceptibility testing results in an early timeframe. Furthermore, recent advancements which can provide direct AST results from positive blood cultures such as Accelerate Phenotest® and Alfred 360® AST system are not yet available in developing countries [19].

However, there are limitations associated with RAST methodology, some unique to resource limited settings. Firstly, the technique is not applicable for polymicrobial infections as well as organisms whose growth rate is slow. Secondly, the technique is labour intensive due to manual readings done at 2 hourly intervals, making it difficult to comply with. Thirdly, measuring zone of inhibitions at earlier time points, particularly at 4-hour, is a difficult task due to difficulty in visualizing the edge of bacterial growth and is prone to human errors and subjective interpretation while measurement. Another major challenge of implementing RAST methodology in resource limited settings is to identify isolates before AST results, without which results can't be interpreted. In resource limited settings, isolates are identified either by inoculating biochemicals manually or by automated microbial identification systems. Both require isolated colonies on plated media which usually takes 18-24 hours before identification can be attempted. Hence, any benefit of doing rapid AST is countered by delays in identification of micro-organisms. Studies, which implemented RAST methodology in their workflow, have identified isolates by MALDI-TOF after short incubation on solid media [16,17]. It is available in only few centres in developing countries, hence implementing RAST in resource limited settings is challenging without it. However, other novel approaches may help to circumvent this limitation by facilitating timely identification of micro-organisms in such settings. These include direct inoculation of

manual biochemicals from pellets obtained by centrifuging the blood-broth mixture from positive BC bottle or even inoculating cards of Automated bacterial identification systems from the pellet [20,21]. Studies have shown excellent correlation for organism identification, particularly in Gram-negatives, where identification cards were directly inoculated from the pellet [20,21].

Our study has many limitations as it is done in a single centre and number of isolates included for analysis is low. Secondly, we included only bottles showing Gram-negative micro-organisms as they are most prevalent pathogens for sepsis in Indian settings after Coagulase negative *Staphylococcus* (CoNS) spp. [22]. Amongst Gram positive micro-organisms, interpretive criteria are available only for *Staphylococcus aureus*, *Enterococcus faecalis* and *E. faecium* by RAST method. These micro-organisms constitute a low proportion amongst isolates in blood stream infections in Indian settings [22]. As RAST methodology is labour intensive, we wanted to avoid antimicrobial susceptibility testing by RAST methodology for a large number of isolates, interpretive criteria for whom are not available. Thirdly, we couldn't demonstrate any benefit in reduction of TAT for AST as we couldn't assign an interpretive category to AST results due to delays in micro-organism identification due to conventional techniques. Thus, effect of RAST on escalation or de-escalation of antibiotic therapy was not assessed in the present study. Fourthly, we didn't correlate RAST results with various resistance mechanisms, prevalent in Gram negative pathogens. Furthermore, our results should be interpreted in context of resistance profile of isolates obtained. Hence, we suggest conducting further studies addressing these limitations and correlation with patient outcomes and antimicrobial usage is required to explore the full potential of this technique.

Conclusions

There is an urgent need to provide timely AST results in sepsis to initiate appropriate antimicrobial therapy, early in the course of disease. Many newer technologies which aim to fulfill this objective are currently unavailable in resource limited settings. Thus, augmentation of existing conventional methodologies may be a practical approach. Development of RAST methodology by EUCAST is a much-needed step in right direction and ideally suited for resource limited settings. By implementing it in routine workflow, TAT can be reduced considerably. This will help in improving outcomes, timely escalation or de-escalation of antibiotic therapy. However, the methodology is labour intensive as it requires readings every 2 hours. Hence, we suggest reading and reporting of results at a single, most accurate time point, which is 8-hour, in our study. However, identification of micro-organisms within this timeframe of 8 hours is a challenge, particularly for resource limited settings. Large scale multicentric studies, with impact on clinical outcomes are needed to realize the full potential of the RAST methodology.

Authors' Contributions

Mohammad Aadam Bin Najeeb: Conceptualization, Performance of test method, Acquisition of data, Software entry, Writing-Original draft Preparation. Ayush Gupta: Conceptualization, Supervision, Data Analysis, Reviewing and Editing of Final draft. Shashank Purwar: Conceptualization, Supervision, Reviewing and Editing of Final draft. Vishnu Teja Nallapati: Performance of test method, Acquisition of data, Writing-Original draft Preparation. Jogender Yadav: Performance of test method, Acquisition of Data, Software entry, Data Analysis, Writing-Original draft Preparation. Farha Siddiqui: Software entry, Data Analysis, Writing-Original draft preparation. All authors approve the final draft of the submitted manuscript.

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