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Original Article

Microbiology Section

# Performance of Rapid Antibiotic Susceptibility Testing from Semiautomated Blood Culture Bottles with Disk Diffusion using CLSI Guidelines: A Cross-sectional Study

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## ABSTRACT

**Introduction:** With currently available microbiological diagnostic tools, the Turnaround Time (TAT) of a positive Blood Culture (BC) report with conventional Antimicrobial Susceptibility Testing (AST) takes 5-9 days, delaying treatment. With Rapid Antimicrobial Susceptibility Test (RAST), a positive BC can be reported within 4-8 hours. Semiautomated ColorCult BC bottles have a chemical sensor at the bottom that continuously detects the increase in carbon dioxide produced by microbial growth. As of today, the RAST method has been validated only for automated systems, not for semiautomated culture systems.

**Aim:** To evaluate the performance of RAST from semiautomated BC bottles with disk diffusion.

**Materials and Methods:** This cross-sectional study was conducted at Hassan Institute of Medical Sciences, Hassan, Karnataka, India from February 2024 to August 2024 and included 144 positively flagged semiautomated BC bottles (Microxpress ColorCult vial, India) showing monomicrobial growth on Gram stain. These bottles were collected and processed for both RAST and standard AST. Results obtained in RAST at 4, 6 and 8 hours were correlated with the standard AST using Clinical and Laboratory Standards Institute (CLSI) M100 as the gold standard. Equivalence criteria, according to Food and Drug Administration (FDA), were: Categorical Agreement (CA) ≥90%, very major error (vmj) ≤1.5% and major error (maj) ≤3%.

**Results:** Among 144 positively flagged semiautomated BC bottles, 53 (36.8%) showed monomicrobial growth. *Escherichia coli* (n=18, 34%), *Klebsiella pneumoniae* (n=16, 30.2%), *Acinetobacter baumannii* (n=9, 17%), *Pseudomonas aeruginosa* (n=5, 9.4%) and *Staphylococcus aureus* (n=5, 9.4%) were isolated. Among all antibiotic-organism combinations tested, CA for RAST at 4-, 6- and 8-hour readings were 80.5% (252/313), 87.4% (384/439) and 91.0% (491/540), respectively. Vmj rates were 5.2% (8/152), 2.95% (7/238) and 1.64% (5/306); maj rates were 6.08% (7/115), 4.8% (7/160) and 1.65% (3/193). Among all antibiotics tested, poor CA was noted for amikacin, tobramycin, piperacillin-tazobactam and ciprofloxacin at all reading times.

**Conclusion:** RAST performed with semiautomated BC bottles at eight hours is equivalent to standard disk diffusion using CLSI guidelines, with a marginal VME rate.


**Keywords:** Antimicrobial susceptibility testing, Blood stream infections, Disk diffusion antimicrobial test

## INTRODUCTION

Bloodstream Infections (BSIs) can cause a range of systemic symptoms, including sepsis, septic shock and organ failure in affected individuals [1]. The mortality rate associated with BSIs in hospitalised patients ranges from 14-37% [2]. The primary diagnostic approach involves isolating the responsible organism from the blood sample and conducting AST for that organism [2]. Prompt initiation of appropriate antimicrobial therapy improves outcomes in sepsis, as each hour of delay is associated with a 9% increase in the odds of mortality [3].

Using currently available microbiological diagnostic methods, the TAT for a positive blood culture report with conventional AST is approximately 7-9 days, while semiautomated methods take about 5-7 days [3]. This delays initiation of definitive treatment by clinicians. To combat this delay, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has introduced RAST, which allows rapid assessment of antimicrobial susceptibility directly from positively signalled blood culture bottles within 8 hours [4]. The RAST method offers specific breakpoints for assessments at 4, 6 and 8 hours of incubation [5,6].

Semiautomated culture bottles (Microxpress Colorcult vial, India) are used for the qualitative assessment of microorganisms from blood and other bodily fluids, as opposed to fully automated systems. Each vial features a chemical sensor at the bottom that continuously detects the increase in carbon dioxide produced by microbial growth. A positive colour change in the vial suggests the presumptive presence of live multiplying microorganisms [7].

However, EUCAST RAST has been validated using blood culture bottles for BACTEC (Beckton Dickinson), BacT/ALERT (bioMérieux) and VersaTREK (Thermo Fisher), but not for semiautomated Colorcult bottles (Microxpress). The reliance on conventional AST and automated AST methods has caused delays in initiating definitive treatment, resulting in extended hospital stays and higher rates of morbidity and mortality. The introduction of a rapid antimicrobial susceptibility test could shorten TAT, facilitate timely administration of appropriate antibiotics and potentially reduce fatalities.

The advantages of semiautomated culture bottles (Microxpress Colorcult vial, India) over automated systems include that they do not require specialised equipment, results can be visualised by eye, making interpretation accessible to laboratory technicians and they are economically feasible. Validating the use of RAST in semiautomated culture bottles would enable earlier positive BC reporting, even in low-resource settings, helping financially constrained patients benefit from timely results. This study proposes to address the lack of data on validating semiautomated culture systems (Microxpress Colorcult vial, India) for RAST with conventional methods.

## MATERIALS AND METHODS

This cross-sectional study was conducted in the clinical microbiology laboratory of Hassan Institute of Medical Sciences, Hassan,

Karnataka, India from February 2024 to August 2024. Institutional Ethical Committee clearance was obtained for the study (IEC Reference number: IEC/HIMS/RR 617/04-10-2024).

**Inclusion criteria:** Isolates with monomicrobial growth on Gram stain were included in the study.

**Exclusion criteria:** Isolates with polymicrobial growth on Gram stain were excluded from the study.

**Estimation of sample size:** Based on a pilot study (out of 628 samples, 257 were positively flagged with growth; 257×100/628=41%), the prevalence of positive BC samples was 41%. The sample size was calculated using the formula $N=4pq/d^2$. The critical value ZA/2 for 95% confidence (1.96) was used. p=0.41 and q=1-p=0.59. A relative precision of 20% of p was employed, i.e., d=0.2×0.41=0.082. Based on these values, the required sample size was 144. Samples from the pilot study were not included in the current study.

### Study Procedure

**Sample collection and processing:** The study was carried out on 144 semiautomated BC bottles that were positively flagged and demonstrated monomicrobial growth on Gram staining, either Gram-positive or Gram-negative. These samples were collected and analysed using both RAST and standard AST methods. The study included only one isolate from each patient.

**Rapid Antimicrobial susceptibility test (RAST) [5,6]:** RAST was performed according to the guidelines. Lawn culture was prepared by inoculating each 90-mm circular MH/MH-F agar plate with 125±25 µL of undiluted BC suspension from a positive BC bottle, using a swabbing technique in three directions. Antibiotic disks were applied in accordance with established methods for AST protocols, with a maximum of six disks per plate to prevent interference between agents. Inoculated plates were incubated immediately and readings were taken at 4 hours, 6 hours and 8 hours. Antibiotic disks used were Ampicillin (AMP) 10 µg; amoxicillin-Clavulanic Acid (AMC) 20/10 µg; Piperacillin-tazobactam (PTZ) 100/10 µg; Cefotaxime (CTX) 5 µg; Ceftazidime (CAZ) 10 µg; Imipenem (IPM) 10 µg; Meropenem (MEM) 10 µg; Ciprofloxacin (CIP) 5 µg; Levofloxacin (LEV) 5 µg; Amikacin (AMK) 30 µg; Gentamicin (GEN) 10 µg; Tobramycin (TOB) 10 µg; Trimethoprim-Sulfamethoxazole (TMP-SMX) 1.25/23.75 µg; Cefepime (CFP) 30 µg; Clindamycin (CLI) 2 µg; Cefoxitin (FOX) 30 µg; Norfloxacin (NOR) 10 µg.

**RAST [5,6] and CLSI-M100 (33TH ed) [8]:** CLSI M100 is acknowledged as the reference guideline for this study, as CLSI guidelines are routinely followed for AST in our laboratory. The outcomes obtained from RAST at 4 hours, 6 hours and 8 hours were analysed in comparison to those generated by the conventional CLSI methodology. Results classified within the Area of Technical Uncertainty (ATU) in RAST were omitted from evaluation, following the recommendations outlined in the RAST guidelines. In present study, 34 drug-bug combinations at 4 hours, 29 at 6 hours and 23 at 8 hours were classified as ATU.

CA was established when the interpretive category for a specific drug-bug combination was concordant between both methodologies. Vmj occurs when the standard AST result is resistant (R) while the RAST result is susceptible (S). Maj is defined as a situation in which the standard AST result is susceptible (S) and the RAST result is resistant (R). Min is identified when the reference result is intermediate (I) and the RAST result is resistant (R) or susceptible (S) [3]. The overall CA rates and error rates were evaluated based on the acceptable standards established by FDA acceptance criteria and CLSI M52 standards. Specifically, the requirements stipulate that CA must be ≥90%, vmj should be <1.5% and maj must be ≤3% [Table/Fig-1] [9,10].

## STATISTICAL ANALYSIS

Descriptive statistics were used to analyse concordance (CA), with results expressed as frequencies and percentages. Errors were classified as vmj, maj, or min [9,10] and their distributions were summarised accordingly.

| Category | New method (RAST) | Reference method (disk diffusion) | Formulas for the calculation of the errors |
|---|---|---|---|
| Categorical Agreement (CA) | R | R | - |
| Categorical Agreement (CA) | S | S | - |
| very major error (vmj) | S | R | Vmj discrepancies<br>Total resistant drug: bug combinations by disk diffusion |
| major error (maj) | R | S | maj discrepancies<br>Total susceptible drug: bug combinations by disk diffusion |
| minor error (min) | S/R | I | min discrepancies<br>Total drug: bug combinations tested |

**[Table/Fig-1]:** Criteria used to analyse RAST against AST.

## RESULTS

Among 144 positive-flagged BC bottles, 53 (36.8%) showed monomicrobial growth: *E. coli* (n=18, 34.0%), *Klebsiella pneumoniae* (n=16, 30.2%), *Acinetobacter baumannii* (n=9, 17.0%), *Pseudomonas aeruginosa* (n=5, 9.4%) and *Staphylococcus aureus* (n=5, 9.4%) [Table/Fig-2]. Of all the antibiotics tested, the CA of rapid AST at 4-, 6- and 8-hour reading times was 80.5% (252/313), 87.4% (384/439) and 91.0% (491/540), respectively. Vmj rates were 5.2% (8/152), 2.95% (7/238) and 1.64% (5/306), whereas maj rates were 6.08% (7/115), 4.38% (7/160) and 1.65% (3/193).

| Isolates | Number of isolates (n=53) |
|---|---|
| *E .coli* | 18 (34%), |
| *Klebsiella pneumoniae* | 16 (30.2%), |
| *Acinetobacter baumannii* | 9 (17%) |
| *Pseudomonas aeruginosa* | 5 (9.4%) |
| *Staphylococcus aureus* | 5 (9.4%) |

**[Table/Fig-2]:** Isolates identified in the study.

The acceptable standards by FDA and CLSI M52 were equivalent for RAST at eight hours of incubation with standard disk diffusion. At eight hours of incubation, the RAST method exhibited vmj rates in *E. coli* isolates for gentamicin, amoxicillin-clavulanic acid and ciprofloxacin, while *Klebsiella pneumoniae* showed vmj for meropenem and tobramycin. Maj rates were observed in *Klebsiella pneumoniae* for meropenem, *Acinetobacter baumannii* for piperacillin-tazobactam and *Pseudomonas aeruginosa* for cefepime [Table/Fig-3].

| Isolates | Very major error (Vmj ≤1.5%) | Major error (maj ≤3%) |
|---|---|---|
| *E.coli* | 1 GEN<br>1 AMC<br>1 CIP | 0 |
| *Klebsiella pneumoniae* | 1 MRP<br>1TOB | 1 MRP |
| *Acinetobacter* | 0 | 1 PTZ |
| *Pseudomonas* | 0 | 1 CPM |

**[Table/Fig-3]:** Drug: bug combination with very major error (vmj) and major error (maj) rates at eight hours of incubation.

Based on the acceptable standards established by FDA acceptance criteria and CLSI M52, CA must be ≥90%, vmj should be <1.5% and maj must be ≤3% [9,10].

At six hours, CA was observed in 168 of 188 *E. coli*-antimicrobial combinations tested. Four combinations showed vmj, two showed maj. Fourteen combinations showed min. At four hours, CA was observed in 119 of 144 *E. coli*-antimicrobial combinations tested. Three combinations showed vmj, two showed maj, 20 combinations showed min [Table/Fig-4].

| Organisms -Total isolates | 4 hours | | | | 6 hours | | | | 8 hours | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CA | Vmj | Maj | Min | CA | Vmj | Maj | Min | CA | Vmj | Maj | Min |
| *E. coli* - 18 | 119/144 (82.6%) | 3/73 (4.10%) | 2/51 (3.9%) | 20/144 (13.8%) | 168/188 (89.3%) | 4/114 (3.5%) | 2/60 (3.3%) | 14/188 (7.4%) | 205/224 (91.5%) | 3/139 (2%) | 0/69 (0) | 16/224 (7.1%) |
| *K. pneumoniae* - 16 | 96/119 (80.7%) | 3/64 (4.6%) | 2/37 (5.4%) | 18/137 (13.3%) | 131/153 (85.6%) | 2/100 (2%) | 2/35 (5.7%) | 18/153 (11.7%) | 167/186 (89.7%) | 2/102 (1.9%) | 1/68 (1.4%) | 16/186 (8.6 %) |
| *A. baumannii* - 9 | 31/40 (77.5%) | 1/20 (5%) | 2/14 (14.28%) | 6/46 (13%) | 44/49 (90.5%) | 0/13 (0) | 1/32 (3.1%) | 4/49 (8.1%) | 62/68 (91.1%) | 0/26 (0) | 1/37 (2.7%) | 5/68 (7.3%) |
| *P. aeruginosa* - 5 | * | * | * | * | 29/34 (85.3%) | 1/13 (7.7%) | 2/19 (10.5%) | 2/38 (5.2%) | 38/42 (90.4%) | 0/13 (0) | 1/26 (3.8%) | 3/42 (7.1%) |
| *S. aureus* - 5 | 6/10 (60%) | 1/4 (25%) | ¼ (25 %) | 2/10 (20%) | 12/15 (80%) | 0/6 (0) | 0/6 (0) | 3/10 (30%) | 19/20 (95%) | 0/13 (0) | 0/6 (0) | 1/20 (10%) |

**[Table/Fig-4]:** Comparison of RAST with routine AST methodology in terms of microorganisms.

[Table/Fig-5] For PTZ at four hours of incubation, concordance was noted for 21 PTZ-bacteria combinations and the CA for PTZ was calculated as 21/26 (80.8%). The vmj rate was 1/14 (7.1%), calculated as the number of susceptible isolates (1) by RAST divided by the number of resistant isolates (14) by the standard disk diffusion method. The maj rate was 1/9 (11.1%), calculated as the number of resistant isolates (1) by the RAST method divided by the number of susceptible isolates (9) by standard disk diffusion. The min rate was 3/26 (11.5%), calculated as the number of isolates classified as intermediate by RAST but as susceptible or resistant by standard disk diffusion. The table can be interpreted similarly for the rest of the antibiotics at different hours of incubation.

## DISCUSSION

Present study assessed the RAST methodology using semiautomated culture bottles in accordance with EUCAST and employing disk diffusion based on CLSI guidelines. When applying standard criteria for equivalence, specifically CA ≥90%, vmj ≤1.5% and maj ≤3%, the RAST conducted at eight hours demonstrated equivalence to the standard disk diffusion method, achieving CA

| Antibiotics | 4 hours | | | | 6 hours | | | | 8 hours | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CA | Vmj | Maj | Min | CA | Vmj | Maj | Min | CA | Vmj | Maj | Min |
| PTZ | 21/26 (80.76%) | 1/14 (7.14%) | 1/9 (11%) | 3/26 (11.5%) | 28/34 (82.3%) | 1/17 (5.8%) | 2/14 (14.2%) | 3/34 (8.8%) | 34/39 (87.1%) | 0/17 0 | 1/18 (5.55%) | 4/39 (10.25%) |
| LE | 26/30 (86.6%) | 1/15 (6.6%) | 0/11 0 | 4/30 (1.33%) | 38/42 (90.42%) | 1/19 (5.2%) | 1/21 (4.7%) | 2/38 (5.26%) | 42/46 (91.3%) | 0/19 0 | 0/23 0 | 4/46 (8.6%) |
| AMC | 20/25 (80%) | 2/20 (10%) | 0/2 0 | 3/25 (12%) | 25/27 (92.5%) | 1/22 (4.5%) | 0/4 0 | 1/17 (5.88%) | 33/35 (94.28%) | 1/29 (3.5%) | 0/5 0 | 1/35 (2.8%) |
| CAZ | 10/17 (58.8%) | 1/6 (16.5%) | 1/6 (16.5%) | 5/17 (29.4%) | 29/33 (87.8%) | 0/18 0 | 1/12 (8.3%) | 3/33 (9%) | 37/40 (92.5%) | 0/24 0 | 0/13 0 | 3/40 (7.5%) |
| AMP | 4/5 (80%) | 0/2 0 | 0/2 0 | 1/5 (20%) | 8/10 (80%) | 0/4 0 | 0/4 0 | 2/10 (20%) | 10/11 (90%) | 0/5 0 | 0/5 0 | 1/11 (9%) |
| COT | 26/30 (86.6%) | 1/17 (5.8%) | 0/10 0 | 3/30 (10%) | 29/33 (87.8%) | 1/25 (4%) | 0/5 0 | 3/33 (9%) | 34/37 (92%) | 0/25 0 | 0/10 0 | 2/3 (66.6%) |
| MRP | 22/27 (81.4%) | 0/9 0 | 2/15 (13%) | 3/27 (11.1%) | 33/37 (89.1%) | 1/20 (5%) | 1/14 (7.1%) | 3/37 (8.1%) | 38/43 (88.3%) | 1/25 (4%) | 1/15 (6.6%) | 3/43 (9.3%) |
| CIP | 17/22 (77.2%) | 1/9 (11.1%) | 0/9 0 | 4/22 (18.1%) | 35/40 (87.5%) | 1/28 (3.5%) | 0/8 0 | 4/40 (10%) | 41/46 (89.1%) | 1/32 (3.3%) | 0/10 0 | 4/46 (8.6%) |
| TOB | 16/21 (76.1%) | 0/10 0 | 0/6 0 | 5/21 (23.8%) | 29/33 (87.8%) | 1/18 (5.5%) | 0/12 0 | 3/33 (9%) | 43/47 (91.4%) | 1/30 (3.4%) | 0/14 0 | 3/47 (6.3%) |
| CTX | 22/26 (84.6%) | 0/10 0 | 1/14 (7.1%) | 2/26 (7.6%) | 25/27 (92.6%) | 0/10 0 | 0/15 0 | 2/27 (7.4%) | 31/33 (93.9%) | 0/14 0 | 0/17 0 | 2/33 (6%) |
| AK | 20/27 (74%) | 0/10 0 | 0/10 0 | 7/27 (26%) | 27/34 (79.4%) | 0/17 0 | 0/10 0 | 7/34 (20.5%) | 41/47 (87.2%) | 0/24 0 | 0/18 0 | 5/47 (10.6%) |
| IPM | 23/27 (85.1%) | 0/12 0 | 1/12 (8.3%) | 3/27 (11.12%) | 35/41 (85.3%) | 0/12 0 | 1/25 (4%) | 4/41 (9.7%) | 42/46 (91.3%) | 0/17 0 | 0/25 0 | 4/46 (8.6%) |
| GEN | 17/20 (85%) | 0/10 0 | 0/7 0 | 3/20 (15%) | 24/27 (96%) | 0/17 0 | 0/7 0 | 3/27 (11.1%) | 44/47 (93.6%) | 1/33 (3%) | 0/11 0 | 3/47 (6.3%) |
| CPM | * | * | * | * | 4/5 (80%) | 0/2 0 | 1/3 (33.3%) | 0/5 (20%) | 4/5 (80%) | 0/1 0 | 1/3 (33.3%) | 1/5 (20%) |
| CX | 1/2 (50%) | 1/2 (50%) | 0 | 0 | 3/4 (75%) | 0/2 0 | 0/1 0 | 1/4 (25%) | 5/6 (83.3%) | 0/3 0 | 0/2 0 | 1/6 (16%) |
| NX | 3/3 (66.6%) | 0/3 0 | 0 | 0/3 0 | 6/6 (100%) | 0/4 0 | 0/2 0 | 0/6 0 | 6/6 (100%) | 0/5 0 | 0/1 0 | 0/6 0 |
| CD | 4/5 (80%) | 0/3 0 | 1/2 (50%) | 0/5 0 | 6/6 (100%) | 0/3 0 | 0/3 0 | 0/6 0 | 6/6 (100%) | 0/3 0 | 0/3 0 | 0/6 0 |
| Total | 252/313 (80.5%) | 8/152 (5.26%) | 7/115 (6.08%) | 46/313 (14.6%) | 384/439 (87.4%) | 7/238 (2.95%) | 7/160 (4.3%) | 41/439 (9.3%) | 491/540 (91%) | 5/306 (1.6%) | 3/193 (1.6%) | 41/530 (7.7%) |

**[Table/Fig-5]:** Comparison of RAST with routine AST methodology in terms of antibiotics.
CA: Categorical agreement; Vmj: Very major error; Maj: Major error; Min: Minor error; PTZ: Piperacillin tazobactam; LE: Levofloxacin; AMC: Amoxicillin clavulanic acid; CAZ: Ceftazidime; AMP: Ampicillin; COT: Cotrimoxazole; MRP: Meropenem; CIP: Ciprofoxacin; TOB: Tobramycin; CTX: Cefotaxime; AK: Amikacin; IPM: Imipenem; GEN: Gentamicin; CPM: Cefepime; CX: Cefoxitin; NX: Norfloxacin; CD: Clindamycin
*CPM is tested only for *Pseudomonas* and zone range is not available in RAST for 4 hours.

of 91% (491/540), vmj rates of 1.64% (5/306) and maj rates of 1.65% (3/193).

All studies published evaluating the RAST in conjunction with EUCAST/CLSI guidelines have utilised advanced identification systems such as VITEK or MALDI-TOF for organism identification and the execution of AST. The reliance on these sophisticated methods poses challenges for implementation in low-resource settings [11]. As ours is a resource-limited setting, organism identification on the same day was challenging and was performed using conventional methods. In low-resource settings, commonly used lower-tier, narrow-spectrum antibiotics covering all species among Gram-negative and Gram-positive organisms in RAST are analysed and if sensitive, antibiotic therapy can be initiated immediately without waiting for the final culture report [12].

At eight hours, CA was achieved in 205 of 224 *E. coli*-antimicrobial combinations. Specifically, three *E. coli*-drug combinations demonstrated vmj, two exhibited maj and 16 showed min [Table/Fig-4]. These results contrast with the findings reported by Bin Najeeb MA et al., in their study, where *E. coli* did not attain CA, instead presenting with two maj, three vmj and 13 min [3].

In the present study, poor CA of RAST with CLSI guidelines was noted for PTZ, MRP, CIP, AK, CPM and CX at eight hours of incubation. PTZ and CIP showed poor CA even at eight hours, similar to the research conducted by Bin Najeeb MA et al., [3]. Beta-lactams penetrate bacterial cells through porins to target the cell wall synthesis pathway. The discrepancies may have arisen from inhibition of this translocation due to the presence of blood components in the inoculum [13]. Similarly, porins serve as the primary route for hydrophilic antibiotics, such as fluoroquinolones, to penetrate the bacterial outer membrane [14].

In the context of AMK resistance, the low CA was attributed to vmj, with the highest incidence of these errors observed in *Klebsiella pneumoniae*. This may be related to the organism's dense mucoid growth, as a direct blood broth inoculum was used [2,12]. In a study performed by Akerlund A et al. and Bianco G et al., antibiotics tested against *Staphylococcus aureus* and *Pseudomonas* aeruginosa showed good CA [15,16]. However, in the present study, poor CA for CPM and CX were noted and may be attributed to the smaller number of *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates in our sample. Unlike the studies by Bin Najeeb MA et al., Cherkaoui A et al. and Choudhari CS et al., we found poor CA for MRP. In meropenem resistance, vmj and maj were observed, resembling the study conducted by Bin Najeeb MA et al., [3]. The majority of MRP min results were caused by *Klebsiella pneumoniae*, which may reflect technical errors in the initial phase of the study or variations in drug diffusion caused by dense growth [2], or technical errors in interpreting the zone diameters or the selection of heteroresistant phenotypes during the preliminary stage [12]. Present study observed that most errors occurred with β-lactam drugs and aminoglycosides, similar to the study carried out by Imtiaz A et al., [17]. Among the discordant results in our research, min rates were elevated at all time points. This may be attributed to slight discrepancies in zone breakpoints between EUCAST and CLSI, highlighting the need for harmonisation between them [3,18].

RAST has a few limitations. First, not all the zones were available within four hours [19]. Interpreting zone diameters in the early hours of incubation presents challenges due to thin and faint growth and the edges of the zones may be hazy. The potential for error is heightened, as even a 1 mm variation in measurement can significantly impact results. The margins between susceptible and resistant categories are less discernible [20].

### Limitation(s)

Present study was conducted in a low-resource setting, which posed challenges due to the absence of an automated system for isolate identification prior to performing AST. Furthermore, the study was limited to BCs exhibiting monomicrobial growth, as RAST is validated only for such samples, thereby excluding polymicrobial growth. Owing to time constraints, authors were unable to evaluate the clinical impact of antibiotic escalation and de-escalation. Additionally, the scarcity of studies comparing RAST performance using semiautomated (MicroXpress ColorCult vial, India) BC bottles further limits the generalisability of present study findings.

## CONCLUSION(S)

RAST performed with semiautomated ColorCult BC bottles at eight hours is equivalent to the standard disk diffusion method performed using CLSI guidelines, with a marginal maj rate. From present study, it is evident that RAST can be implemented even in low-resource settings without automated equipment. Establishing a RAST system will help clinicians initiate antibiotic therapy earlier, thereby improving patient care.

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