Content available at: https://www.ipinnovative.com/open-access-journals

IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

Agreement between AST from minute colony (8-10h growth) and mature colony (16-18h/overnight incubation)

Sonali Padhy[1], Ketan Priyadarshi[2], Sarumathi Dhandapani[3], Stessy Ann Punnen[1], Apurba S Sastry[1]*

[1]*Dept. of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India*
[2]*Dept. of Microbiology, All India Institute of Medical Sciences, Patna, Bihar, India*
[3]*Dept. of Microbiology, Sri Devaraj Urs Medical College, Kolar, Karnataka, India*

ARTICLE INFO

Article history:
Received 05-08-2024
Accepted 22-08-2024
Available online 29-09-2024

Keywords:
Sepsis
ASTTAT
minute colony
mature colony

ABSTRACT

Background: Sepsis is a medical emergency where a successful patient outcome depends on early and appropriate antibiotic treatment. This study was conducted to evaluate agreement between antimicrobial susceptibility testing (AST) from minute colonies that can reduce the AST-TAT by as much as 8-10h as compared to the CLSI recommended protocol of performing AST from an overnight (16-18h) growth of mature colony.

Materials and Methods: In the present study, mDD results from minute colony (8-10h growth) were compared to the rDD results mature colony (16-18h/overnight incubation) CA and various types of errors were evaluated.

Results: 237 pathogens and 1597 organism-antibiotic combinations were evaluated, there was a CA of 93.30% which was extremely satisfactory and categorical disagreement was found only in 4.56% of organism-antibiotic combinations, which were mainly mE (4.56%) with nil VME (0%) and ME (0%).

Conclusion: We have found that minute colony (8-10h) AST is in agreement with reference mature colony (16-18h) AST, shortening TAT by (8-10h) earlier than the conventional reference method which is very helpful in treatment of sepsis patients.

1. Introduction

Sepsis is a medical emergency currently defined as "life-threatening organ dysfunction caused by a dysregulated host response to infection."[1] It is a seven global health concern, with an estimated 11 million fatalities linked to it annually out of 48.9 million cases contributing to 20% of all deaths globally. This is more than 20 deaths every minute.[2] Although sepsis is a global illness, its prevalence is highest in low- and middle-income nations due to a lack of treatment resources. Hospitalized individuals with sepsis have a higher death rate, which is thought to be between 20 and 30 percent.[3] Treatment for sepsis can be lifesaving. High-quality clinical care is required for this, particularly in primary care clinics and hospitals with operation, critical, and emergency rooms. Furthermore, a successful patient outcome depends on early and appropriate antibiotic treatment.

A clinical microbiologist's primary goal should be to provide patients with the best treatment possible by rapid turnaround time of blood culture reports.[4] Employing automated blood culture systems instead of conventional culture, sending at least two sets of culture before giving the first dose of antibiotics, collecting the recommended volume of blood aseptically for blood culture, and promptly

* *Corresponding author*.
E-mail address: drapurbasastry@gmail.com (A. S. Sastry).

https://doi.org/10.18231/j.ijmmtd.2024.038

reporting the Gram stain report of positive blood culture bottles are a few strategies to shorten the turnaround time for blood culture reports.[5] Performing antimicrobial susceptibility testing (AST) from minute colonies can reduce the AST-TAT by as much as 8-10h as compared to the CLSI recommended protocol of performing AST from an overnight (16-18h) growth of mature colony. However, agreement of minute-colony AST with mature colony-AST has never been evaluated so far.

Therefore, we conducted this study to determine the agreement of minute-colony AST with mature colony-AST has never been evaluated so far.

2. Materials and Methods

Minute colony Antimicrobial susceptibility test (mDD) was performed according to CLSI guideline by Kirby -Bauer's disk diffusion test.[6] After 8-10 hours of colony growth about 3-5 fresh colonies from a non-selective medium like blood agar are touched to make a direct suspension in sterile normal saline. Turbidity is adjusted to 0.5 McFarland standard which is then lawn cultured onto Mueller Hinton Agar (MHA) by rotating the plate at an angle of 60° for 3 times within 15 minutes. Then the agar plates were dried for 2-5 minutes, and the antibiotic disks of appropriate panel were as per the organism identification were applied on to the MHA surface, using sterile forceps. The same procedure was followed for reference mature colony disk diffusion test (rDD) after 16-18 hours/overnight incubation of colony growth. The zone diameters were measured using a Vernier Caliper and interpreted as per CLSI breakpoints, after 16-18 hours of incubation according Clinical and Laboratory Standards Institute (CLSI) guidelines.[7] Along with this a control plate of ATCC Escherichia coli 25922 and ATCC *Staphylococcus aureus* 25923 were also subjected to AST to ensure the quality of the antimicrobial disks used.

2.1. Study design and analysis

mDD results were compared to the rDD results from the subculture plates. Categorical agreement (CA) was evaluated, using breakpoints mentioned in CLSI M100 ED33-2023, following the exclusion of any antibiotics to which the pathogen is known to have intrinsic resistance.

A panel of eight antibiotics was tested on Gram-negative bacteria such as Amikacin 30μg (AK), Ciprofloxacin 5μg (CIP), Ceftriaxone 30μg (CTR), Ceftazidime 30 μg (CAZ), Cefoperazone Sulbactam 75/30μg (CFS), Piperacillin-tazobactam 100/10μg (PIT), and Meropenem 10 μg(MERO), Tigecycline15μg (TIGE), Minocycline 30μg (MINO). If the pathogen was identified to belong to the *Acinetobacter* species or *Enterobacteriaceae* family, then all of these were included for study with the exception of CAZ for members of *Enterobacteriaceae* family. For *Pseudomonas* species CTR was excluded and Aztreonam (AZTR) was included in the analysis. For *Acinetobacter* species CTR and CIP were excluded from analysis. For *Providencia stuartii* TIGE was excluded from analysis. All other non-fermenters were not included in the study's analysis since the antibiotic panel employed in it was different. The antibiotic panel used for *Stenotrophomonas maltophilia* included Levofloxacin, Co-trimoxazole 1.25/23.75μg and Minocycline and for *Salmonella enterica* subsp. enterica Chloramphenicol, Ceftriaxone 30μg (CTR), Ampicillin 10μg (AMP), Co-trimoxazole 1.25/23.75μg (COT) and Ciprofloxacin 5μg (CIP).

The antibiotic panel used for *Staphylococcus aureus* comprised of 7 disks such as cefoxitin 30 μg (OX), erythromycin 15μg (ER), co-trimoxazole 1.25/23.75μg (COT), clindamycin 2μg (CN) ,tetracycline 30μg (TE), levofloxacin 5μg (LE) and linezolid 30μg (LZ) and for Enterococcus species ampicillin 10μg (AMP), high level gentamicin 120μg (HLG), tetracycline 30μg (TE), and linezolid 30μg (LZ), vancomycin 30μg (VAN), minocycline 30μg (MINO).

The minute colony disk Diffusion Test results were compared with the reports of reference mature colony disk diffusion test and the closeness of agreement was analysed, thereby establishing the efficiency of minute colony Disk Diffusion Test from positively flagged blood culture. Comparisons between mDD and rDD's performance were made using categorical disagreement and CA. The categorical disagreement was further characterized into minor error (mE), major error (ME), and very ME (VME) as depicted in (Table 1). Microsoft Excel document contained all of the collected data. IBM SPSS Statistics for Windows, Version 19.0, was used to analyse the data.

Table 1: Terminologies used for comparison of performance of minute colony disk diffusion test with reference disk diffusion tests

CA				Categorical disagreement			
				mE		ME	VME
rDD	S	I	R	R or S	I	S	R
mDD	S	I	R	I	R or S	R	S

R=Resistant, S=Sensitive, I=Intermediate, mE=Minor error, ME=Major error, VME=Very ME, rDD=Reference disk diffusion, mDD=Minute Colony disk diffusion, CA=Categorical agreement

3. Results

During the study period, 237 freshly flagged positive blood cultures from patients suspected of having bloodstream infections (BSIs) were identified by the routine (reference) laboratory method. Antimicrobial susceptibility test made from the minute colony (minute colony-AST or mDD) results were compared to the results of reference mature colony disk diffusion test (rDD) performed from

the subculture plates. Categorical agreement (CA) was evaluated, using breakpoints mentioned in CLSI M100 ED33-2023, following the exclusion of any antimicrobials to which the pathogen is known to possess intrinsic resistance.

[Table 2] shows the distribution of bacteria isolated from the positive blood cultures for which both mDD and rDD tests were performed. GNB accounts for 53.16% (126) of total isolates; and Gram-positive cocci 23.63% (56). Among Gram-negative bacilli, Escherichia coli was the most common isolate (24.47%), followed by Klebsiella pneumoniae (19.41%), *Acinetobacter baumannii* (12.24%), *Pseudomonas aeruginosa* (9.28%), *Enterobacter cloacae* (4.64%), *Salmonella enterica* subsp enterica (4.64%) and *Stenotrophomonas maltophilia* (1.69%). *Staphylococcus aureus* (15.61%) was the most prevalent isolate among Gram-positive cocci followed by *Enterococcus faecium* (7.17%) and *Enterococcus faecalis* (0.84%). They were subjected to further analysis of CA between mDD and rDD.

As shown in Table 3, overall, mDD performed excellent with a CA of 93.30% with rDD; mE of 4.56% and ME, VME both 0% fulfilling the performance criteria , is considered acceptable (ME ≤3%; VME ≤3%). mE were highest in Klebsiella pneumoniae (8.42%).

Upon analyzing the discrepancy in the quantity of antibiotics used in each isolate, it was found that the majority of the organisms exhibited disagreements at <2 antibiotics per isolate (20.20%); significant disagreement at ≥2 antibiotics was observed with Klebsiella pneumoniae (13.04%).

Among Escherichia coli (Table 4), CA was >90% for all the antibiotics in the panel AND 100% for TIGE.VME and ME both were 0%. However higher mE was observed for CF (8.62%) with kappa value of 0.798, CFS (6.90%) with kappa value of 0.830. There was a CA of > 95% in Klebsiella pneumoniae for all the antibiotics tested except for CF (93.48%). mE was high for MINO (23.91%) with kappa value of 0.571 followed by TIGE (17.39%) with kappa value of 0.537.VME and ME both were 0%. For *Enterobacter cloacae*, the CA was 100% for all the antibiotics in the panel except for AK and PIT both (90.91%). And mE was 9.09% for both AK and PIT. However due to lesser number of isolates of *Enterobacter cloacae* statistical significance could not be determined and so kappa value is denoted as NA (not available) in the (Table 4).

Among *Pseudomonas aeruginosa* (Table 5), CA was 100% for all the antibiotics in the panel except CAZ and PIT (95.45%) and CFS (90.09%). VME and ME both were 0%. However higher mE was observed for CFS (9.09%).

For *Acinetobacter baumannii* (Table 6), the CA was 100%% for CFS, MINO with >95% for all other antibiotics in the panel except for TIGE (89.66%). mE was high for TIGE (10.34%) with kappa value of 0.284.

Among other GNB like Salmonella enterica subsp. enterica and *Stenotrophomonas maltophilia* CA was 100% for all antibiotics tested although statistical significance could not be determined due to lesser number of isolates.

Among Gram-positive cocci, *Enterococcus faecalis* had CA of 100%. *Staphylococcus aureus* (Table 7) had CA of 100% for all antibiotics tested except TE (97.29%) and both ER, LEVO (89.19%). Higher mE of 10.81% was observed for both ER and LEVO. *Enterococcus faecium* (Table 8) had CA of 100% for all the antibiotics tested except HLG and MINO (94.12%).

Statistical significance was determined with p value < 0.001 for Escherichia coli, Klebsiella pneumoniae, *Acinetobacter baumannii*, *Staphylococcus aureus* for all the antibiotics tested and not for other organisms as the number of isolates were insufficient for statistical analysis. The data was analysed using SPSS software version 19.0.

4. Discussion

Developing novel diagnostic methods for antimicrobial susceptibility is one of the goals of the Global Action Plan on Antimicrobial Resistance of the World Health Organization, which aims to decrease bacterial resistance.[8] According to a research by Baltas et.al. in 2020, patients who received efficient antibiotic therapy early had a better chance of surviving than those who did not, with the latter group ultimately facing a larger risk of mortality.[9] Additionally, this study demonstrated that antimicrobial resistance was frequently the cause of treatment failure, highlighting the necessity of faster ASTs. Published research to date has demonstrated the need of treating patients with BSI with antimicrobials as soon as possible to lower death rates and hospital expenses associated with these infections.[10]Therefore, it is crucial to provide rapid diagnostic techniques to identify the microorganisms causing BSI as well as early implementation of targeted antimicrobial therapy which is considered as one of the crucial stewardship intervention. Our study was done to determine the agreement of minute-colony AST with mature colony-AST which are the first of its kind research work, which has never been studied to the best of our knowledge. This would reduce the AST-TAT by 8-10h.

In the present study, we evaluated 237 pathogens and 1597 organism-antibiotic combinations. Overall, there was a CA of 93.30% which was incredibly satisfactory.[Table 3] The categorical disagreement was found only in 4.56% of organism-antibiotic combinations, which were mainly mE (4.56%) with nil VME (0%) and ME (0%). (Table 3) Percentages of errors (mE, ME, and VME) were overall much lower than the acceptable performance criteria of International Standard ISO 20776-2 (ME ≤3%; VME ≤3%). There are paucity of recent literature comparing mDD with rDD, as most of the studies focused on the comparison of mDD with AST from colonies by automated

Table 2: Distribution of bacteria isolated from positive blood cultures for which both mDD and rDD were performed

Organisms	**Number of isolates tested, n (%)**
GNB	126(53.16%)
Escherichia coli (*E. col*i)	58(24.47%)
Klebsiella pneumoniae (*K. pneumoniae*)	46(19.41%)
Enterobacter cloacae (*Ent.cloacae*)	11(4.64%)
Salmonella enterica subsp. enterica	11(4.64%)
Pseudomonas aeruginosa	22(9.28%)
Acinetobacter baumannii	29(12.24%)
Stenotrophomonas maltophilia	4(1.69%)
GPC	56(23.63%)
Staphylococcus aureus	37(15.61%)
Enterococcus faecium	17(7.17%)
Enterococcus faecalis	2(0.84%)
Total	237

Table 3: Performance of mDD compared to rDD for various groups of organisms

Organisms and antibiotics tested (n×Ab=N)	**CA, (n%)**	**Categorical disagreement, n (%)**					
		Among isolate-antibiotic combinations tested				**Among the isolates tested**	
		Minor error	**Major error**	**Very Major error**	**Total**	**Disagreement at <2 antibiotics**	**Disagreement at ≥2 antibiotics**
Escherichia coli(58x7=406)	95.3% (387/406)	4.91% (19/406)	0%	0%	4.91% (19/406)	22.41% (13/58)	5.17% (3/58)
Klebsiella pneumoniae (46x7= 322)	92.24% (297/322)	8.42% (25/322)	0%	0%	8.42% (25/322)	28.26% (13/46)	13.04% (6/46)
Enterobacter cloacae (11x7=77)	97.40% (75/77)	2.67% (2/77)	0%	0%	2.67% (2/77)	18.18% (2/11)	0%
Salmonella enterica subsp. enterica (11 x 5=55)	100% (55/55)	0%	0%	0%	0%	0%	0%
Pseudomonas aeruginosa (22 x7=154)	97.40% (150/154)	2.67% (4/154)	0%	0%	2.67% (4/154)	18.18% (4/22)	0%
Acinetobacter baumannii (29 x 7=203)	96.55% (196/203)	3.57% (7/203)	0%	0%	3.57% (7/203)	17.24% (5/29)	3.45% (1/29)
Stenotrophomonas maltophilia (4 x3=12)	100% (12/12)	0%	0%	0%	0%	0%	0%
Staphylococcus aureus (37 x 7=259)	96.52% (250/259)	3.6% (9/259)	0%	0%	3.6% (9/259)	24.32% (9/37)	0%
Enterococcus faecium (17 x5=85)	80% (68/85)	2.94% (2/85)	0%	0%	2.94% (2/85)	11.76% (2/17)	0%
Enterococcus faecalis (2x5=10)	100% (10/10)	0%	0%	0%	0%	0%	0%
Overall (1597)	93.30% (1490/1597)	4.56% (68/1597)	0%	0%	4.56% (68/1597)	20.20% (48/237)	2.53% (6/237)

Table 4: Performance of mDD compared to rDD test for Enterobacterales

Antibiotics	Organism	CA, n(%)	Categorical disagreement, n (%)				Kappavalue (95% CI)
			Minor	Major	Very Major	Total	
Amikacin (AK)	*E. coli* (n=58)	55 (94.83)	3(5.17)	0	0	3(5.17)	0.812
	K. pneumoniae (n=46)	45(97.82)	1(2.17)	0	0	1(2.17)	0.957
	Ent. Cloacae (n=11)	10(90.91)	1(9.09)	0	0	1(9.09)	NA
Ciprofloxacin (CF)	*E. coli* (n=58)	53(91.38)	5(8.62)	0	0	5(8.62)	0.798
	K. pneumoniae (n=46)	43(93.48)	3(6.52)	0	0	3(6.52)	0.886
	Ent. Cloacae (n=11)	11(100)	0	0	0	0	NA
Cefoperazone-Sulbactam (CFS)	*E. coli* (n=58)	54(93.10)	4(6.90)	0	0	4(6.90)	0.830
	K. pneumoniae (n=46)	46(100)	0	0	0	0	1
	Ent. Cloacae (n=11)	11(100)	0	0	0	0	NA
Piperacillin-tazobactam (PIT)	*E. coli* (n=58)	55(94.83)	3(5.17)	0	0	3(5.17)	0.812
	K. pneumoniae (n=46)	44(95.65)	2(4.35)	0	0	2(4.35)	0.865
	Ent. Cloacae (n=11)	10(90.91)	1(9.09)	0	0	1(9.09)	NA
Meropenem (MERO)	*E. coli* (n=58)	57(98.28)	1(1.72)	0	0	1(1.72)	0.956
	K. pneumoniae (n=46)	46(100)	0	0	0	0	1
	Ent. Cloacae (n=11)	11(100)	0	0	0	0	NA
Tigecycline (TIGE)	*E. coli* (n=58)	58(100)	0	0	0	0	1
	K. pneumoniae (n=46)	38(82.60)	8(17.39)	0	0	8(17.39)	0.537
	Ent. Cloacae (n=11)	11(100)	0	0	0	0	NA
Minocycline (MINO)	*E. coli* (n=58)	55(94.83)	3(5.17)	0	0	3(5.17)	0.820
	K. pneumoniae (n=46)	35(76.10)	11(23.91)	0	0	11(23.91)	0.571
	Ent. Cloacae (n=11)	11(100)	0	0	0	0	NA

Table 5: Performance of mDD compared to rDD test for *Pseudomonas aeruginosa*

Pseudomonas aeruginosa (n=22)	CA,n (%)	Categorical disagreement, n (%)			
		Minor	Major	Very Major	Total
Ceftazidime (CAZ)	21(95.45)	1(4.55)	0	0	1(4.55)
Ciprofloxacin(CF)	22(100)	0	0	0	0
Cefoperazone-Sulbactam (CFS)	20(90.09)	2(9.09)	0	0	2(9.09)
Piperacillin-tazobactam (PIT)	21(95.45)	1(4.55)	0	0	1(4.55)
Amikacin (AK)	22(100)	0	0	0	0
Meropenem (MERO)	22(100)	0	0	0	0
Aztreonam (AZTR)	22(100)	0	0	0	0

Table 6: Performance of mDD compared to rDD test for *Acinetobacter baumannii*

Acinetobacter baumannii (n=29)	CA, n(%)	Categorical disagreement, n (%)				Kappa value (95% CI)
		Minor	Major	Very Major	Total	
Ceftazidime (CAZ)	28(96.55)	1(3.45)	0	0	1(3.45)	0.858
Piperacillin-tazobactam (PIT)	28(96.55)	1(3.45)	0	0	1(3.45)	0.772
Cefoperazone-Sulbactam (CFS)	29(100)	0	0	0	0	1
Amikacin(AK)	28(96.55)	1(3.45)	0	0	1(3.45)	0.871
Meropenem(MERO)	28(96.55)	1(3.45)	0	0	1(3.45)	0.884
Tigecycline (TIGE)	26(89.66)	3(10.34)	0	0	3(10.34)	0.284
Minocycline(MINO)	29(100)	0	0	0	0	1

Table 7: Performance ofmDD compared to rDD test for *Staphylococcus aureus*

Staphylococcus aureus **(n=37)**	**CA,n (%)**	**Categorical disagreement, n (%)**				**Kappa value (95% CI)**
		Minor	**Major**	**Very Major**	**Total**	
Cefoxitin (OX)	37(100)	0	0	0	0	1
Erythromycin(ER)	33(89.19)	4(10.81)	0	0	4(10.81)	0.806
Clindamycin(CN)	37(100)	0	0	0	0	1
Co-trimoxazole (COT)	37(100)	0	0	0	0	1
Tetracycline (TE)	36(97.29)	1(2.70)	0	0	1(2.70)	0.905
Levofloxacin(LEVO)	33(89.19)	4(10.81)	0	0	4(10.81)	0.806
Linezolid(LZ)	37(100)	0	0	0	0	1

Table 8: Performance of mDD compared to rDD test for *Enterococcus faecium*

Enterococcus faecium **(n=17)**	**CA,n (%)**	**Categorical disagreement, n (%)**			
		Minor	**Major**	**Very Major**	**Total**
Ampicillin (AMP)	17(100)	0	0	0	0
High level gentamicin (HLG)	16(94.12)	1(5.88)	0	0	1(5.88)
Tetracycline (TE)	17(100)	0	0	0	0
Linezolid (LZ)	17(100)	0	0	0	0
Vancomycin (VAN)	17(100)	0	0	0	0
Minocycline (MINO)	16(94.12)	1(5.88)	0	0	1(5.88)

systems (Vitek2, Phoenix, or Micro scan).[11–15]

We conducted a unique analysis of the categorical disagreement at ≤2 and ≥2 antibiotics. We observed that the categorical disagreement at < 2 and ≥2 antibiotics were 20.20% and 2.53%, respectively (Table 3), by which we can derive that even if there is categorical disagreement, majority will have discrepancies with <2 antibiotics.

Among Escherichia coli (Table 4), CA was >90% for all the antibiotics in the panel AND 100% for TIGE. There was a CA of > 95% in Klebsiella pneumoniae for all the antibiotics tested except for CF (93.48%). (Table 4) For *Enterobacter cloacae* (Table 4), the CA was 100%% for all the antibiotics in the panel except for AK and PIT both (90.91%). Among *Pseudomonas aeruginosa* (Table 5), CA was 100% for all the antibiotics in the panel except CAZ and PIT (95.45%) and CFS (90.09%). For *Acinetobacter baumannii* (Table 6), the CA was 100%% for CFS, MINO with >95% for all other antibiotics in the panel except for TIGE (89.66%). *Staphylococcus aureus* (Table 7) had CA of 100% for all antibiotics tested except TE (97.29%) and both ER, LEVO (89.19%). *Enterococcus faecium* (Table 8) had CA of 100% for all the antibiotics tested except HLG and MINO (94.12%). For all the organisms tested mE was less than 10% except for *Acinetobacter baumannii* for TIGE (10.34%), *Staphylococcus aureus* for both ER and LEVO (10.81%) and VME and ME both were 0% which is the most important conclusion of our study. Another significant finding in our study was among other GNB like *Salmonella enterica* subsp. enterica and *Stenotrophomonas maltophilia* and GPC like *Enterococcus faecalis* CA was 100% for all antibiotics tested. To the best of our knowledge, there is no additional literature available to compare the outcomes.

5. Conclusion

In critically ill patients, BSI presents a high risk of morbidity and death. Early intervention with the right antimicrobial drugs combined with supportive care leads to better patient outcomes.[8] Nevertheless, conventional culture methods are laborious, and results are frequently not available for 48 hours following the patient's disease presentation. Early detection and tailored therapeutic intervention for sepsis patients may be possible with prompt identification of bacteria grown in blood cultures along with AST.

6. Ethical Approval

This study was done after taking Ethics committee approval from the Institutional Ethics Committee (Human Studies), JIPMER, Puducherry. REG. NO. EC/NEW/INST/2020/331.

7. Source of Funding

None.

8. Conflicts of Interest

None.

References

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA*. 2016;315(8):801–10.
2. Kempker JA, Martin GS. A global accounting of sepsis. *Lancet*. 2020;395(10219):168–70.

3. World Health Organization. Global report on the epidemiology and burden of sepsis: current evidence, identifying gaps and future directions. Available from: https://www.who.int/publications/i/item/9789240010789.
4. Søgaard M, Nørgaard M, Schønheyder HC. First notification of positive blood cultures and the high accuracy of the gram stain report. *J Clin Microbiol*. 2007;45(4):1113–7.
5. Kirn TJ, Weinstein MP. Update on blood cultures: how to obtain, process, report, and interpret. *Clin Microbiol Infect*. 2013;19(6):513–20.
6. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. *Am Soc Microbiol*. 2009;15(1):1–23.
7. Clinical and Laboratory Standards Institute (CLSI) 2023. Performance Standards for Antimicrobial Susceptibility Testing. 33rd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.
8. World Health Organization. Global action plan on antimicrobial resistance. 2015;Available from: https://www.who.int/publications/i/item/9789241509763.
9. Baltas I, Stockdale T, Tausan M, Kashif A, Anwar J, Anvar J, et al. Impact of antibiotic timing on mortality from Gram-negative bacteraemia in an English district general hospital: the importance of getting it right every time. *J Antimicrob Chemother*. 2021;76(3):813–19.
10. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med*. 2006;34(6):1589–96.
11. Ha J, Hong SK, Han GH, Kim M, Yong D, Lee K, et al. Same-Day Identification and Antimicrobial Susceptibility Testing of Bacteria in Positive Blood Culture Broths Using Short-Term Incubation on Solid Medium with the MicroFlex LT, Vitek-MS, and Vitek2 Systems. *Ann Lab Med*. 2018;38(3):235–41.
12. Tian PP, Su SS, Zhu LS, Wang T, Yang H, Du MY, et al. Short-term culture for rapid identification by mass spectrometry and automated antimicrobial susceptibility testing from positive bottles. *BMC Infect Dis*. 2024;doi:10.1186/s12879-024-09475-x.
13. Tsai YW, Lin TC, Chou HY, Hung HY, Tan CK, Wu LC, et al. Shortening the Time of the Identification and Antimicrobial Susceptibility Testing on Positive Blood Cultures with MALDI-TOF MS. *Diagnostics (Basel)*. 2021;11(8):1514. doi:10.3390/diagnostics11081514.
14. Idelevich EA, Schüle I, Grünastel B, Wüllenweber J, Peters G, Becker K, et al. Rapid identification of microorganisms from positive blood cultures by MALDI-TOF mass spectrometry subsequent to very short-term incubation on solid medium. *Clin Microbiol Infect*. 2014;20(10):1001–6.
15. Mauri C, Principe L, Bracco S, Meroni E, Corbo N, Pini B, et al. Identification by mass spectrometry and automated susceptibility testing from positive bottles: a simple, rapid, and standardized approach to reduce the turnaround time in the management of blood cultures. *BMC Infect Dis*. 2017;17:749. doi:10.1186/s12879-017-2851-5.

Author biography

Sonali Padhy, Junior Resident https://orcid.org/0009-0009-2499-9932

Ketan Priyadarshi, Assistant Professor

Sarumathi Dhandapani, Assistant Professor

Stessy Ann Punnen, Project Officer

Apurba S Sastry, Additional Professor

Cite this article: Padhy S, Priyadarshi K, Dhandapani S, Punnen SA, Sastry AS. Agreement between AST from minute colony (8-10h growth) and mature colony (16-18h/overnight incubation). *IP Int J Med Microbiol Trop Dis* 2024;10(3):219-225.