Antibiotic Susceptibility Testing (AST) Practices in India: Need for a National AST Forum

Kalyani Borde 1 , Manick Dass 1 , Ratna Mani Sharma 2 , Dilip Mathai 3

1. Microbiology, Apollo Institute of Medical Sciences and Research, Hyderabad, IND
2. Microbiology, Apollo Hospitals, Hyderabad, IND
3. Infectious Diseases, Apollo Institute of Medical Sciences and Research, Hyderabad, IND

Corresponding author: Kalyani Borde, k14august@gmail.com

Abstract

Background

Accurate interpretation of antibiotic susceptibility testing (AST) is one of the most crucial functions of the microbiology laboratory. However, its performance depends on a number of critical factors. We conducted a status survey to understand the existing practices in Indian laboratories that have a potential to influence performance of AST.

Method

We developed a 22-point online survey questionnaire on information about respondent’s specifications, use of AST consumables, existing quality control protocols, and matters of contention in AST practices, and sent it by Google forms to 362 clinical microbiologists (holding MD or DNB certification). Participation was voluntary. Results were analyzed using descriptive statistics.

Results

Among 362, a total of 103 returned the questionnaire. The first 100 responses that were complete (all 22 questions answered) were analyzed. Respondents were from medical colleges (61%), private hospitals (26%), and stand-alone laboratories (13%). Analysis revealed that the Clinical & Laboratory Standards Institute (CLSI) guidelines were followed by all. Overall, 54% used disc diffusion as the primary method for performing AST. For the internal quality control testing of media and AST, 24% and 16% had adequate testing components and frequency, respectively. For performing AST of colistin, broth microdilution was used by 19%. Also, 86% participated in external quality control programs, and 54% respondents were dissatisfied or unsure about the development of competencies in AST methodology during their postgraduate training.

Conclusion

This survey reveals that potential gaps exist in the performance parameters and internal quality control of AST. There is an urgent need for harmonization in AST performance and postgraduate training in clinical microbiology in India.
covering important parameters regarding AST methodology as follows:

- Preliminary information about the respondents: (a) type of the laboratory, (b) place, (c) sample load, and (d) experience in years of the respondents.

- Consumables for AST: (a) availability of automation, (b) type of automated system for AST, (c) broth microdilution (BMD) or disk diffusion, (d) information about commercial/in-house media, (e) type of media used for AST of fastidious organisms, (f) BMD for colistin susceptibility testing, and (g) disks used for performing AST.

- Quality control (QC) protocols: (a) components of internal QC testing, (b) QC of media, (c) QC of AST, (d) frequency of QC testing, (e) preparation of inoculum for AST testing, (f) participation in external quality assurance services (EQAS), and (g) guidelines followed for interpretation.

- Respondent’s opinion about matters of contention in AST methodology: (a) whether the microbiologist is consulted before purchasing AST supplies, (b) reason behind selection of AST supplies, (c) requests for AST for antibiotics lacking defined breakpoints, (d) clinical correlation of results, and (e) satisfaction with the training in AST methodology.

As the total number of practicing clinical microbiologists in India is unavailable, we used the directory of life members of IAMM, which enlists 3,600 members. The sample size, accordingly, was calculated as 94 (confidence interval of 95% and margin of error at 10%).

Participation in the survey was voluntary. We shared this questionnaire on Google forms with the clinical microbiologists (n=362). We closed the survey within one month of sharing the questionnaire and analyzed the responses.

**Results**

Out of 362, 105 (27%) agreed to participate in the survey. Of these 105, responses of the first 100 participants who had answered all the 22 questions were analyzed (Table 1).

| Sl. no. | Parameter | Responses | % |
|-------|-----------|-----------|---|
| 1     | Methodology for AST* | Disk diffusion | 71 |
|       |           | Automated AST | 36 |
| 2     | Availability of automation for AST | Yes | 63 |
|       |           | No | 37 |
| 3     | Name of the automated systems for AST | VITEK-2 | 88 |
|       |           | PHOENIX | 11 |
|       |           | MicroScan WalkAway | 1 |
| 4     | Guidelines for interpretation | CLSI | 100 |
| 5     | Participation in EQAS | Yes | 86 |
|       |           | No | 14 |
|       |           | Sterility check of media | 76 |
| 6     | Components of IQC* | Thickness measurement of media | 40 |
|       |           | pH of media | 39 |
|       |           | None | 5 |
|       |           | With each batch of media | 53 |
|       |           | Once a week | 28 |
|       |           | Once a month | 20 |
|   | Frequency of IQC for AST of control strains* | Once in three months | 7 |
|---|--------------------------------------------|----------------------|---|
|   |                                            | Once in six months   | 3 |
|   |                                            | Never                | 12|
|   | Preparation of 0.5 McFarland inoculum      | Use of densitometer  | 33|
|   |                                            | Manual comparison with commercial standards | 29|
|   |                                            | Tentatively          | 20|
|   |                                            | Manual comparison with in-house standards | 18|
|   | Media for AST of fastidious organisms      | 5% sheep blood agar  | 44|
|   |                                            | 5% human blood agar  | 38|
|   |                                            | MHA with 5% sheep blood | 17|
|   |                                            | Soybean casein agar with 5% sheep blood | 1|
|   | BMD for colistin susceptibility            | Yes                  | 19|
|   |                                            | No                   | 81|
|   | Opinion of the microbiologist sought before purchasing consumables for AST | Always               | 68|
|   |                                            | Sometimes            | 14|
|   |                                            | Never                | 18|
|   | Factors affecting purchase decisions*      | Internal validation  | 45|
|   |                                            | Cost                 | 34|
|   | Perception of clinical correlation of AST results | No major discrepancies perceived | 47|
|   |                                            | Unsure about clinical correlations due to lack of feedback | 46|
|   |                                            | Discrepancies observed often | 5|
|   |                                            | Discrepancies are never observed | 3|
|   | Requests for testing and reporting of antibiotics without defined breakpoints | Yes                  | 39|
|   |                                            | Maybe                | 10|
|   |                                            | No                   | 51|
|   | Satisfaction with the post-graduate training regarding AST methodology | Yes                  | 46|
|   |                                            | Maybe                | 19|
|   |                                            | No                   | 35|

**TABLE 1: Important findings from the survey on antibiotic susceptibility practices in India.**

*More than one response per question was possible.

AST, antibiotic susceptibility testing; EQAS, external quality assurance scheme; IQC, internal quality control; MHA, Mueller-Hinton agar; BMD, broth microdilution; DD, disk diffusion

---

**Preliminary information**

Respondents worked in the laboratory types as those attached to medical colleges (61%), private hospitals (26%), and stand-alone laboratories (13%). They were practicing in Karnataka (30), Telangana (23), Andhra Pradesh (15), Tamil Nadu (8), West Bengal (6), four each in Uttar Pradesh and Maharashtra, three each in...
Kerala and Odisha, two each in Assam and Delhi, and one each in Chhattisgarh and Gujarat. Sample load for culture/susceptibility testing was classified as high (>50 tests daily) for 32% laboratories, moderate (20-50 tests daily) for 47%, and low (<20 tests daily) in the remaining. Participants’ experience ranged from >10 years (38%), between 5 and 10 years (25%), and 1-5 years (37%), after obtaining the certification (MD/ DNB) in microbiology.

Consumables for AST
a. 71% used disc diffusion for AST, while 17% used only automated methods; 17% used a combination of both the methods.

b. The antibiotic discs were obtained from HiMedia laboratories by 87% and from more than one manufacturer by 20%.

c. 96% prepared media for AST in-house from dehydrated powders, and 87% obtained media from HiMedia laboratories.

d. 63% respondents had automation for AST.

e. Of those who had automation, 88% used VITEK®-2 system (bioMérieux, Marcy-l’Etoile, France), 11% used PHOENIX (BD, Sparks, MD), and 1% used MicroScan WalkAway (Dade Behring, Deerfield, IL).

f. For performing AST of fastidious organisms such as Streptococcus, 17% used Mueller–Hinton agar (MHA) with 5% sheep blood, while 38% used human blood agar (Table 1).

g. For colistin susceptibility testing, 19% performed BMD testing.

Quality control protocols
All the respondents used CLSI breakpoints for reporting AST, and 86% participated in EQC programs. Among the four components of internal quality control (IQC) testing of media (Table 1), 30% implemented any one, 26% implemented any two, and 39% implemented three or more components of IQC. No IQC protocols for AST media were implemented by 5% respondents.

IQC was performed with each new batch of media and/or discs by 53% of the respondents, and 16% performed IQC once a week and with each new batch of media and/or discs. No susceptibility testing was performed as a part of IQC by 12% respondents. The frequency of IQC was once a week for 28%, once a month for 20%, once in three months for 7%, and once in six months for 3% of the respondents.

Overall, 33% used a densitometer for preparing 0.5 McFarland of the inoculum, 20% adjusted the inoculum tentatively, while 18% and 29% adjusted the inoculum by manual comparison with in-house and commercial standards, respectively.

Respondent’s opinions
Microbiologist’s opinions were always sought before purchasing consumables according to 68% of the respondents, and 18% and 14% were never or only sometimes consulted for the same, respectively.

Decisions based on quality of the products were made by 45% who also had a system in place for internal validation before making purchase decisions. However, for 34%, cost was the most important factor while selecting the AST consumables. Trust and relations with the manufacturing company were the most important factors affecting purchase decisions for 17%. Subject expertise and troubleshooting support provided by the manufacturer were the most important factors for 4%.

Out of all the respondents, 47% could not be sure whether the AST results correlated clinically due to lack of feedback from the clinicians. Also, 46% stated that there usually are no major discrepancies between laboratory results and clinical response, 5% observed discrepancies often, and 5% never observed such discrepancies.

Testing and reporting antibiotics with no defined breakpoints were requested to 39% of the respondents, while 10% stated that they were maybe approached for the same and 51% never faced this issue.

Of the respondents, 46% were satisfied with the training they received during their post-graduation period regarding AST methodology, 35% were dissatisfied, and 19% were unsure about it.

Discussion
Reliability of AST depends on compliance with the carefully defined standards of performance. The

2022 Borde et al. Cureus 14(11): e30971. DOI 10.7759/cureus.30971
interpretation of AST can vary widely when these standards are not met. This survey among practicing clinical microbiologists throws light on the status of AST performance in our region.

Medical college laboratories from the four states in southern India (n=79) are predominantly represented in this survey. Most respondents have >10 years of experience, closely followed by those with 1-5 years of experience.

One of the key findings from the survey is that the disk diffusion method for performing AST is most commonly used. This points toward the wide adaptation of disk diffusion methodology in India.

Disks as well as media were most commonly obtained from HiMedia laboratories, with other suppliers being Microexpress®, Oxoid™, and Bio-Rad laboratories. A study comparing the performance of antibiotic disks from various manufacturers found wide variations in the results when using same disks from different manufacturers [7]. Similarly, wide variation was observed in the performance of the dehydrated media from a few manufacturers [8]. No such study exists from India, severely restricting the ability of practicing microbiologists to make informed choices. A study from 2008, comparing three manufacturers, also highlighted the issues with variation of results [9]. A study from Nigeria demonstrated poor performance and variation in antibiotic content of locally manufactured disks as against imported disks [10]. Hence, it can be argued that there is a need to establish a reference laboratory for guiding practicing microbiologists in the country regarding the quality of commercially available supplies.

For automated AST, VITEK®-2 was the most commonly used instrument. Automated AST systems are increasingly being adapted by microbiologists in the country as they are easier to perform, are less labor-intensive, and provide quicker results. However, it is also important to note that most of these systems give extrapolated minimum inhibitory concentrations (MICs) and not the true MICs [11,12]. A national reference laboratory is needed to continuously monitor the antibiotic panels and performance of automated systems.

The main concern emerging from this survey is the lack of uniformly adapted quality control protocols for performing AST. Around half of the respondents performed inadequate components with infrequent IQC checks. A significant proportion of the respondents adjusted the inoculum tentatively or with crude methods. Also, only a minor proportion of respondents used appropriate media for performing AST of fastidious organisms, and a large proportion used routine blood agars. A disturbing reality is the extensive usage of human blood agar, which is not recommended by any methodology or performance standards.

All the respondents performed susceptibility testing of colistin, an important last-resort drug in the settings with high prevalence of carbapenem resistance. However, only a small proportion used the recommended method of BMD for the same, questioning the appropriateness of susceptibility results of this important antibiotic. Khurana et al. concluded in a single-center study from India that VITEK®-2 for colistin susceptibility testing is unreliable and suboptimal [13], necessitating wider adaptation of BMD.

A vast majority of the respondents opined that they are never or only sometimes consulted before making purchase decisions about the AST supplies. Also, less than half the respondents had a system of internal validation in place before making purchase decisions. This is an important area for improvement.

An indispensable role of the clinical microbiologist is correlating the AST results clinically. Almost half of the respondents could not comment about this due to lack of feedback from the clinicians. A case can be built to impart inter-professional communication skills among the clinical microbiologists as well as the clinicians if AST is to serve its purpose of guiding therapy.

In a survey by Anand et al., it was shown that more than 90% of the antibiotic FDCs (cefdorico) were irrational [14]. There are no breakpoints available for these drugs. Along these lines, it is prudent for the clinical microbiologist to abstain from reporting such drugs. A significant proportion of the respondents agreed to being approached for testing and reporting such antibiotics for which breakpoints are not available. Under such circumstances, it is imperative that a national forum for AST decides which antibiotics can and cannot be reported in India.

It has become important to inculcate a clinical approach among the microbiologists in the country. More than half of the respondents were dissatisfied or unsure about the quality of training they received during their post-graduation studies. The experience in years of those who expressed dissatisfaction was almost evenly distributed between those with >10, 5-10, and 1-5 years. This again demonstrates a need to train young as well as older adults in this profession. As Wattal pointed out in 2018, the microbiology curriculum has not changed in the past 40 years, and also recommended major changes to train clinical microbiologists [15].

Limitations of the study include a probable biased sampling overrepresenting the microbiologists from the southern states and the government medical college establishments in the country. However, the public health and teaching hospital facilities are the ones needing immediate attention, given their dual role in reaching out to the vast underserved population of the country and training of future microbiologists.
Around 30 nations in the world, including China and South Africa, have constituted national AST committees in recent years. India, too, needs a guiding forum, which combines the expertise of microbiologists, infectious disease physicians, and pharmacologists, for harmonization of AST practices in this country. Its functions could be, but not limited to, those suggested in Table 2[16].

| Suggestions |
|-------------|
| 1. To formulate susceptibility testing strategies at a national level. |
| 2. To encourage quality assurance and to inform and support quality assurance agencies and accreditation programs. |
| 3. To set up a website tailored to national needs in AST methodology |
| 4. To provide education on AST methodology and interpretation. |
| 5. To ensure adequate funding for AST methodology standardization. |
| 6. To investigate and generate data to support breakpoints in comparison with CLSI/EUCAST in the Indian context. |
| 7. To communicate with manufacturers of susceptibility testing material and devices. |
| 8. To provide a technical representative for the international breakpoint committees. |

**TABLE 2: Suggested functions of a national forum for advising and training of the clinical microbiologists in the AST methodology.**

Adapted from EUCAST: suggestions on how to organize and form a NAC[16].

CLSI, Clinical & Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing

**Conclusions**

To summarize, major gaps exist in the IQC, supplies, and training in AST methodology in microbiology laboratories in our region. There is an urgent need to streamline the performance of inarguably the most important function in clinical microbiology laboratory - AST. A national AST committee could fill in the gaps by providing guidance and leadership to the microbiologists. Stakeholders and experts from multiple disciplines should undertake the difficult task of harmonization of AST performance in the country.

**Additional Information**

**Disclosures**

**Human subjects:** Consent was obtained or waived by all participants in this study. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

**References**

1. M100. Performance Standards for Antimicrobial Susceptibility Testing, 32nd Edition . (2022). Accessed: October 12, 2022: https://clsi.org/standards/products/microbiology/documents/m100/.
2. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0 . (2022). Accessed: June 1, 2022: https://www.eucast.org/clinical_breakpoints.  
3. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 12.0 . (2022). Accessed: June 1, 2022: https://aurosan.de/images/media/bek_herb servicematerial/EUCAST_ReStaemme_Sollwerte.pdf.  
4. Microbiology & Virology CMC EQAS. (2022). Accessed: June 1, 2022: http://www.microbiology.iammekscmc.org/.
5. Wattal C, Oberoi JK, Goel N, Datta S, Raveendran R, Prasad KJ: Experience of Indian association of medical microbiology external quality assurance scheme centre, New Delhi: challenges and quality assessment of clinical microbiology laboratories. Indian J Med Microbiol. 2019, 37:163-72. 10.4103/ijmm.IJMM_19_556  
6. Member directory. (2022). Accessed: August 21, 2022: http://www.myiamm.in/MemberArea/MemberDirectory.  
7. Åhman J, Matuschek E, Kahlmeter G: The quality of antimicrobial discs from nine manufacturers-EUCAST evaluations in 2014 and 2017. Clin Microbiol Infect. 2019, 25:546-52. 10.1016/j.cmi.2018.05.021
8. Åhman J, Matuschek E, Kahlmeter G: EUCAST evaluation of 21 brands of Mueller-Hinton dehydrated media for disc diffusion testing. Clin Microbiol Infect. 2020, 26:e1-5. 10.1016/j.cmi.2020.01.018

9. Joshi A, Iyer V, Balasubramaniam U, Kagel A, Bharadwaj R: Comparison of efficacy of three commercially available antibiotic discs. Indian J Med Microbiol. 2008, 26:160-2. 10.4103/0255-0857.40535

10. Eze PM, Ajaegbu EE, Ejikeogu PC, Eghuna RN, Abba CC, Esimone OC: Evaluation of the quality of commercial antibacterial discs available in Nigeria. British J Pharm Res. 2014, 4:2548-62. 10.9734/BJPR/2014/10900

11. Kerr J: Antibiotic treatment and susceptibility testing. J Clin Pathol. 2005, 58:786-7. 10.1136/jcp.2005.030411

12. Matuschek E, Brown DF, Kahlmeter G: Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clin Microbiol Infect. 2014, 20:O255-66. 10.1111/1469-0691.12573

13. Khurana S, Malhotra R, Mathur P: Evaluation of Vitek®2 performance for colistin susceptibility testing for Gram-negative isolates. JAC Antimicrob Resist. 2020, 2:dlaa101. 10.1093/jacard/dlaa101

14. Anand P, Kaur N, Verma V, Shafiq N, Malhotra S: Assessment of rationality of available fixed dose combinations of antibiotics in India. Expert Rev Anti Infect Ther. 2022, 20:797-808. 10.1080/14787210.2022.2015524

15. Wattal C: I.AMM recommended modification of MD microbiology curriculum to MD clinical microbiology as a speciality of medicine under consideration of MCI and Niti Ayog: time has come to move on! Are we ready? Indian J Med Microbiol. 2018, 36:453-7. 10.4103/ijmm.IJMM_19_69

16. Suggestions on how to organise and form a NAC . (2022). Accessed: June 1, 2022: https://www.eucast.org/organization/nac/.