



RESEARCH ARTICLE

Strengthening Antimicrobial Stewardship via Diagnostic Stewardship Education

Dr. Sangeeta Sharma, MBBS, MD (Pharmacology), MBA(HCA)^{1*}, Dr. Renu Gupta, MBBS, MD Microbiology, MPH(MMU), PGDHQM², Dr. Anup Warriar, DM (Infectious Diseases)³, Dr. Sumit Rai, MBBS, MD Microbiology⁴, Dr. Vikas Manchanda, MBBS, MD Microbiology⁵, Dr. Shukla Das, MBBS, MD Microbiology⁶, Dr. Ekta Gupta, MBBS, MD Microbiology⁷, Dr. KK Chopra, MBBS, MD Chest & TB⁸

¹Professor and Head, Institute of Human Behaviour and Allied Sciences, Delhi, INDIA

²Assistant Professor, Institute of Human Behaviour and Allied Sciences, Delhi, INDIA

³Lead Consultant, Infectious Diseases, Aster DM Healthcare, Kochi, Kerala, INDIA

⁴Professor & Head Microbiology, All India Institute of Medical Sciences (AIIMS), Mangalagiri, Andhra Pradesh, INDIA

⁵Professor Microbiology, Maulana Azad Medical College, New Delhi, INDIA

⁶Professor & Head Microbiology, University College of Medical Sciences, Delhi, INDIA

⁷Professor & Head Virology, Institute of Liver and Biliary Sciences, New Delhi, INDIA

⁸Former Director, New Delhi TB Centre Delhi, INDIA



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ABSTRACT

Background: Antimicrobial resistance (AMR) is a global health challenge requiring an integrated approach that includes antimicrobial, infection prevention and control, and diagnostic stewardship. Diagnostic uncertainty drives antibiotic overuse. Diagnostic stewardship enhances accurate diagnoses by optimizing test selection, reducing unnecessary antibiotic use, and improving surveillance, ultimately refining prescribing practices and patient outcomes. Despite diagnostic stewardship's potential to curb inappropriate prescribing and healthcare costs, its adoption remains limited due to low awareness and misconceptions among clinicians.

Methods: This cross-sectional pre-post study engaged 458 multidisciplinary participants to assess awareness regarding DS practices. An online educational intervention comprising five modules covered pre- and post-analytic practices, test interpretation, and the integration of diagnostic stewardship principles within antimicrobial stewardship (AMS) strategies.

Results: Out of 458 participants, 111 participants completed all tests in the five modules. There was a significant increase in knowledge, with mean test scores rising from 29.22 ± 5.80 to 35.45 ± 6.18 ($p < 0.001$) post-intervention. Overall knowledge of antimicrobial stewardship improved substantially, with mean correct responses rising from 58% pre-test to 71% post-test. Antimicrobial resistance awareness increased from 83% to 90%, while report interpretation saw the greatest gain (43% to 63%), followed by AMS strategies (61% to 75%), pre-analytical processes (44% to 56%) and AMS actions (61% to 73%). However, some gaps remained in understanding the limitations of the Widal test, indications for respiratory cultures, redundant antibiotic cover, inappropriate drug-pathogen combinations, and the need to avoid unnecessary antimicrobials in asymptomatic bacteriuria.

Conclusion: The educational intervention led to marked improvements in both core awareness and applied stewardship competencies, especially enhancing appropriate test selection, result interpretation, and dispelling key diagnostic misconceptions. To sustain these gains and drive enduring behavioral change, regular refresher training, seamless integration of diagnostic stewardship into broader antimicrobial and infection prevention and control programs and periodic curriculum updates are essential.

Keywords: Antimicrobial resistance, antimicrobial stewardship, diagnostic stewardship, educational intervention, rational prescribing, pre-analytical and post-analytical microbiological testing practices, Clinical decision-making.

Introduction

Antimicrobial resistance (AMR) poses a serious global health threat, leading to increased morbidity, mortality, and healthcare costs¹. Addressing this crisis necessitates the widespread implementation of antimicrobial stewardship (AMS) to curb the emergence, selection, and spread of AMR pathogens, thereby limiting adverse economic impacts^{1,2}. However, AMS is most effective when supported by infection prevention and control and by diagnostic stewardship, because antibiotic decisions are often made under diagnostic uncertainty³.

Diagnostic stewardship refers to coordinated, evidence-based interventions that promote the right test for the right patient at the right time, with correct specimen collection, timely reporting, and accurate interpretation that informs clinical action. Accurate and timely diagnosis plays a pivotal role in battling AMR, by directly shaping antibiotic initiation, escalation or de-escalation, and duration thus strategically reducing inappropriate AMU^{4,5}. Diagnostic uncertainties arising from underutilized or misused services, coupled with insufficient attention to preanalytical factors, significantly contribute to the inappropriate AMU as clinicians may prescribe empirically for longer than needed or miss opportunities to narrow therapy⁴. Overuse of diagnostic tests or indiscriminate testing leads to excessive and often irrelevant results leading to unnecessary antibiotic exposure, avoidable adverse events, and increased costs, while underuse delays diagnoses or treatment^{6,7,8}. Yet, on the other hand, the suboptimal utilization of microbiology laboratory services and misinterpretation of results underscore the need for improved diagnostic testing and stewardship practices^{8,9,10}.

The diagnostic process is inherently complex, encompassing test selection, meticulous management of pre-analytical, analytical, and post-analytical factors, and evidence-based decision-making^{10,11,12}. Breakdowns at any stage can trigger diagnostic error and inappropriate antimicrobial use. Common examples include treating blood culture contaminants as true bacteremia or ordering urine cultures in the absence of urinary symptoms, both of which can drive avoidable antibiotic use. Yet, in routine care these steps are frequently influenced by habit, time pressure, limited feedback on test quality, and weak

clinician–laboratory coordination. Test selection should consider disease probability, diagnostic accuracy, cost, and proper specimen handling. However, these factors are often overlooked, leading to unnecessary or low-value tests and mismanagement of false-positive results^{9,11}. Advancements in diagnostic testing, such as point-of-care tests (POCT) and molecular tools, hold great potential to reduce unnecessary AMU by shortening time to appropriate therapy and reduce unnecessary antibiotic use, their benefits depend on clear indications, workflow integration, clinician training, and stewardship oversight to prevent misuse and misinterpretation^{13,14,15}.

DS enhances accurate diagnoses by optimizing test selection, reducing unnecessary antibiotic use, and improving surveillance, ultimately refining prescribing practices and patient outcomes. Effective diagnostic stewardship necessitates multidisciplinary collaboration among clinicians, including infectious diseases specialists, intensivists, and clinical microbiologists, to ensure appropriate test utilization and optimize patient care. DS prioritizes basic high-impact diagnostics while regulating the use of novel tests, particularly in low-resource settings. Despite its potential to reduce inappropriate AMU and healthcare costs, DS adoption remains slow due to low clinician awareness, lack of standardized protocols, and challenges in implementation^{12,16}.

In India, diagnostic capacity and utilization vary widely across facilities, and gaps in pre-analytical practices, clinician education, and standardized testing pathways can undermine the contribution of microbiology and rapid diagnostics to AMS. While some tertiary centers have begun integrating diagnostic stewardship into stewardship programs, adoption remains inconsistent due to limited awareness, absence of locally adapted protocols, and operational constraints. Because diagnostic testing is a key driver of antibiotic prescribing, understanding current knowledge and practices is essential for designing scalable stewardship interventions. Education is a pragmatic first step because many stewardship failures reflect modifiable knowledge and decision-process gaps rather than lack of tests alone.

This study aims to assess current knowledge, diagnostic practices, and prevalent misconceptions regarding diagnostic stewardship and evaluates

the effectiveness of an educational intervention in enhancing the application of DS principles within AMS. By strengthening diagnostic decision-making and interpretation, the intervention aims to support more judicious antibiotic use and improved clinical care.

Methods:

STUDY DESIGN: A cross-sectional pre-post study design.

PARTICIPANTS: Clinicians from all over India were invited through email and social media channels to participate in the training program.

EDUCATIONAL INTERVENTION: The course consisted of a comprehensive five-day program, delivered through live online interactive sessions, each lasting three hours.

The focus was primarily on pre- and post-analytical factors, with analytical phase considerations limited to delays in sample processing, reporting, and to interpretation of advanced tests such as PCR and molecular diagnostics as they can lead to misinterpretation of antimicrobial susceptibility, inappropriate therapy, and increased reliance on empirical treatment. This approach was designed to accommodate clinicians from various specialties, excluding core analytical techniques specific to microbiology.

Five modules were designed to enhance doctors' understanding and application of diagnostic tests

to optimize antimicrobial use and patient care. The modules underwent an external peer-review process by a group of experts to ensure quality and relevance.

Module 1 highlighted the magnitude and urgency of acting to combat AMR with focus on the importance of pre-analytical requisites, such as criteria for test selection, sampling methods, storage, transport and quality assessment in common clinical syndromes. Module 2 delved into advantages and limitations of various available microbiological tests including conventional, rapid and molecular tests. Module 3 addressed the interpretation of culture and antibiotic susceptibility test (AST) results, key resistance mechanisms, and the role of minimum inhibitory concentration (MIC), breakpoints, and pharmacokinetics/pharmacodynamics (PK/PD) in optimizing diagnostic testing. Module 4 used clinical vignettes to illustrate the application of diagnostic evidence in antimicrobial selection, the principles of escalation and de-escalation, and the management of infections in critically ill patients. Module 5 discussed implementing stewardship interventions and monitoring and evaluation of the effectiveness of these interventions. Table 1 provides an overview of common stewardship challenges or pitfalls, their impact, and best practices addressed during the programme to enhance antimicrobial prescribing, reinforcing the importance of DS in achieving these goals.

Table 1. Common pitfalls in Diagnostic Stewardship and Strategies /Best Practices for Antimicrobial Stewardship Integration

Stewardship Challenges/pitfalls	Impact	Strategies/Best Practices
Pre-analytical		
Undertesting (e.g., no blood cultures in sepsis, no urine culture in complicated UTI, missing PCR following positive serology)	Missed diagnosis, delayed treatment, increased resistance	Ensure appropriate testing based on clinical guidelines.
Over testing (e.g., unnecessary cultures in asymptomatic bacteriuria, "pan" blood cultures, Syndromic PCR panels in pneumonia)	Unnecessary antibiotic use, false-positive results	Use decision-support tools for appropriate test orders. Restrict testing to clinical indications (e.g., high-risk or symptomatic patients) Implement reflex testing; culture only with pyuria or clinical suspicion.
Improper specimen collection (e.g., contamination, inadequate volume)	False positives/negatives, repeat testing, treatment delays	Train staff, use proper collection techniques, and follow SOPs

Stewardship Challenges/pitfalls	Impact	Strategies/Best Practices
Delayed specimen transport (e.g., improper storage, long transit time)	Bacterial overgrowth or loss of viability, inaccurate results	Timely transport, refrigeration for unsterile sites, use of transport media
Analytical		
Lack of standardization in reporting standards (e.g., variable AST reporting)	Misinterpretation of susceptibility, inappropriate therapy	Use standardized guidelines (CLSI/EUCAST)
Delayed laboratory processing (e.g., batch processing delays)	Slower diagnosis, delayed targeted therapy	Optimize lab workflow, implement rapid diagnostic tests
Limited access to advanced diagnostics (e.g., lack of PCR/molecular testing)	Delayed pathogen identification, empirical therapy reliance	Strengthening lab capacity, integrating molecular methods
Post-analytical		
Inaccurate reporting or misinterpretation (e.g., differentiating colonizers, contaminants and pathogens, incorrect AST interpretation)	Inappropriate therapy Suboptimal antibiotic choice, therapeutic failure	Improve report clarity, implement lab-physician collaboration
Delayed communication of critical results (e.g., sepsis culture reports not relayed urgently)	Treatment delays, increased mortality	Implement rapid reporting systems, direct clinician communication
Lack of integration with AMS programs (e.g., lab results not guiding therapy changes)	Prolonged broad-spectrum use, resistance development	Link microbiology with AMS teams, ensure regular feedback

Synchronous chatting, online polling and question & answer sessions were used to enhance the engagement of the participants. The program received accreditation from the Delhi Medical Council.

Pre- and post-session evaluations were conducted for each module, comprising a total of 50 questions (10 per module), to assess baseline awareness and practices, as well as to measure the impact of the session through score changes. The pre and post-test were developed, administered and analyzed in accordance with the Checklist for Reporting Results of Internet E-Surveys (CHERRIES) to ensure a comprehensive assessment of participants' awareness and understanding¹⁷. Participants received immediate feedback with answers and explanations upon submission of their post-test.

DATA ANALYSIS: Descriptive statistics, including frequency, percentage, mean, and standard deviation, were calculated. Pre- and post-test scores, paired using unique identifiers, were compared using the Wilcoxon Rank Test for non-parametric distributions in IBM SPSS Statistics version 21. Only participants who submitted both pre- and post-tests over the 5-day period were included in the analysis. The Mann-Whitney U test was used to evaluate the relationship between demographic variables (between independent groups) and baseline scores. A p-value of less than 0.05 was considered as significant.

Results:

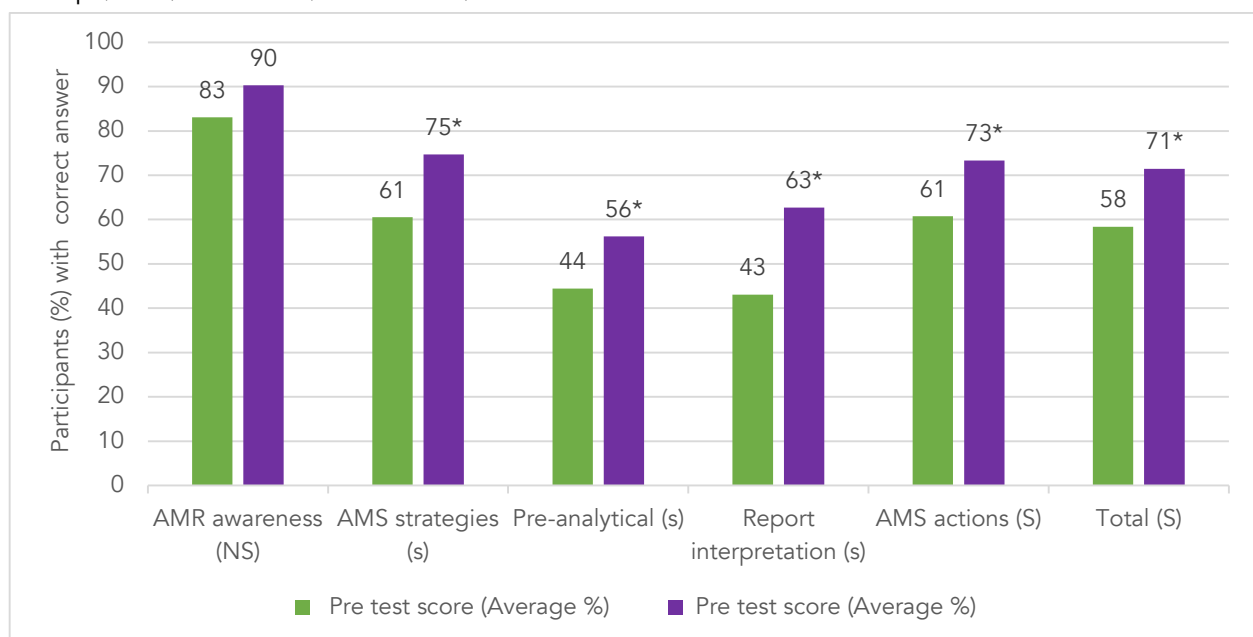
Out of a total of 458 clinicians attending the training program, although an average of 160 participants took either pre and/or post-test daily, 111 clinicians completed both pre-post-tests on all five days. Table 2 depicts the characteristics of the participants who completed pre-post on all days. Professionals from all over the country from different specialties - general medicine, infectious diseases, critical care/anesthesiology, pulmonology, microbiology, and pharmacology participated. Most participants (over 60%) were senior professionals with postgraduate qualifications and more than 5 years' experience, primarily serving in tertiary care hospitals. Microbiologists, senior professionals, and those with over 5 years of experience demonstrated a significantly higher baseline knowledge when compared to their less experienced counterparts, including other clinicians, residents, and professionals with under five years of experience.

Table 2: Participants' profile categorized according to designation, specialization, qualifications, experience, healthcare sector and level of healthcare

Characteristics		N=111 (%)	P value
Designation	Faculty/consultant/specialist	69 (62.16)	0.039
	Medical officers	18 (16.22)	
	Resident doctors	24 (21.62)	
Specialization	Microbiology	35 (31.53)	0.024
	Clinical broad specialties	76 (68.47)	
Qualification	Post-graduate	98 (88.29)	0.388
	Graduate	13 (11.71)	
Years of experience	> 5 years	68 (61.26)	0.001
	<5 years	43 (38.74)	
Healthcare sector	Private	46 (41.44)	0.163
	Public	65 (58.56)	
Healthcare facility level	Tertiary care hospital	96 (86.49)	0.460
	Secondary & primary care	15 (13.51)	

The pre-test mean scores improved significantly from 29.22 ± 5.80 out of 50 (median 31, range 17-44) to 35.45 ± 6.18 (median 38, range 21-47) in

post-test ($p < 0.001$). Figure 1 depicts the percentage of participants answering correctly in the pretest and posttest.

Figure 1: Pre- and post-test scores of participants across key antimicrobial resistance (AMR) and antimicrobial stewardship (AMS) domains (Scores in %)

* $P < 0.05$

The pre- and post-test results (Table 3) demonstrated improvement in participants' knowledge and understanding across various themes following the educational intervention, with the greatest gains observed in areas with lower baseline scores. Analysis of the incorrect response patterns revealed that several misconceptions among participants stemmed from commonly selected distractors in multiple-choice questions. Baseline awareness of AMR principles including its definition, drivers, impact, and

strategies for combating AMR was relatively high (83.06%) and improved further post-intervention (90.27%). Similarly, prior knowledge of multidisciplinary AMS committees, single-dose surgical antimicrobial prophylaxis, antibiograms, antibiotic policies, specimen collection from Foley's catheter, pneumonia treatment duration, limiting catheter use, and the importance of basic IPC measures was already good and showed further enhancement after training.

Following the intervention, participants showed marked improvements in key specimen-handling practices. The percentage correctly identifying indications for urine culture in catheterized patients rose from 27.0% to 45.1%. Adherence to proper blood culture collection methods improved significantly from 49.6% to 67.6%, and correct storage of delayed specimens increased from 63.1% to 83.8% (all $p < 0.001$). Recognition of unacceptable culture specimens and appropriate timing of collection also improved significantly (63.1% to 86.5% and 45.1% to 59.5%, respectively; $p < 0.001$). Post-intervention, there was a slight decline in participants' knowledge regarding the importance of minimizing urinary catheter duration (from 96.4% to 94.6%, $p = 0.31$), though overall awareness remained high.

However, knowledge gaps persisted regarding appropriate test selection, with minimal progress in understanding indications for blood and respiratory cultures (33.3% to 38.8%), serological testing for undifferentiated fever (25.3% to 32.4%), and *C. difficile* testing (25.23% to 32.43%). There was no significant change in knowledge about urine specimen collection methods (87.4% to 83.8%, $p = 0.26$).

In culture and sensitivity interpretation, scores improved significantly. Recognition of clinically significant bacteriuria using colony count thresholds improved from 41.4% to 77.5% ($p < 0.001$). Participants were better at avoiding unnecessary antibiotics based on urine pus cell counts (46.0% to 75.7%), selecting antibiotics using AST reports (35.1% to 69.4%), and using

biomarkers appropriately (22.5% to 57.7%), all with $p < 0.001$. Ability to differentiate colonizers or contaminants from true pathogens also improved (46.0% to 75.7%, $p < 0.001$). Interpretation of PCR results improved moderately (56.8% to 67.6%, $p = 0.01$). While interpretation of PCR results saw a moderate but significant improvement (56.8% to 67.6%, $p = 0.01$), more advanced domains - molecular detection of resistance genes, rapid diagnostic test interpretation, respiratory culture colonizer identification, and avoidance of incorrect drug-bug pairings in AST reports, showed only modest, non-significant changes.

Clinical decision-making also saw progress as therapeutic choices improved in managing conditions like acute diarrhea, Methicillin-Sensitive *Staphylococcus aureus* (MSSA) infections, sepsis, and acute pharyngitis. However, challenges remained in selecting diagnostic tests for undifferentiated fevers, identifying correct indications for cultures, and interpreting *C. difficile* testing. Deficiencies were also evident in report interpretation, particularly detecting errors, overlapping antibiotic coverage, and responding to *Candida* in respiratory samples. Lastly, diagnostic overdependence (e.g., Widal test use for enteric fever) remained a persistent issue. Participants also demonstrated enhanced understanding of broader antimicrobial stewardship concepts, including AMR data application, audit and feedback utilization, selective reporting, formulary restrictions, and the AWARe classification system.

Table 3: Question wise Pre-posttest percent scores across key antimicrobial resistance (AMR) and antimicrobial stewardship (AMS) domains

Intent of the questions	Participants with Correct answer (n=111)				
	Pre-test (n)	Pre-test (%)	Post-test (n)	Post-test (%)	P value
Theme 1: Antimicrobial Resistance (AMR) Awareness					
Understanding of AMR definition.	97	87.39	98	88.29	0.50
AMR as a Natural Biological Phenomenon.	96	86.49	99	89.19	0.27
Strategies for combating AMR	56	50.45	87	78.38	0.00
Optimizing Antibiotic Duration to Combat AMR	104	93.69	105	94.59	0.50
Impact of overuse of antibiotics	103	92.79	105	94.59	0.38
Practices Contributing to AMR	95	85.59	96	86.49	0.50
AMR as a Societal Problem.	100	90.09	103	92.79	0.29
Poor Infection Prevention & Control Contributing to AMR	91	81.98	97	87.39	0.11
Urgency of Addressing AMR	110	99.10	106	95.50	0.11
Use of Antimicrobial Use & AMR data in Combating AMR	70	63.06	106	95.50	0.00

Intent of the questions	Participants with Correct answer (n=111)				
	Pre-test (n)	Pre-test (%)	Post-test (n)	Post-test (%)	P value
Average	92	83.06	100	90.27	0.11
Theme 2: Pre-Analytical Practices					
Correct Urine Specimen Collection Method	97	87.39	93	83.78	0.26
Proper Blood Culture Collection Method to optimize yield	55	49.55	75	67.57	0.00
Unnecessary Testing and Antibiotic Use	30	27.03	50	45.05	0.00
Appropriate Specimen Storage during Delays	70	63.06	93	83.78	0.00
Indications for Blood Culture	37	33.33	43	38.74	0.06
Indications for Respiratory Cultures:	28	25.23	36	32.43	0.14
Recognition of Unacceptable Specimens for Culture	70	63.06	96	86.49	0.00
Optimal Timing for Blood Culture	50	45.05	66	59.46	0.00
Timing for Serological tests in Acute Undifferentiated Fever for correct interpretation of the test	28	25.23	36	32.43	1.14
Indications for <i>C. difficile</i> Testing.	28	25.23	36	32.43	1.14
Average	49	44.41	62.4	56.22	0.01
Theme 3: Report Interpretation					
Significant Colony Count threshold in Bacteriuria	46	41.44	86	77.48	0.00
Clinical Approach to Pyuria	51	45.95	84	75.68	0.00
Choosing Antibiotic from AST Report	39	35.14	77	69.37	0.00
Clinical Interpretation of Serological Biomarkers in Infection Diagnosis & Antimicrobial Therapy Decisions	25	22.52	64	57.66	0.00
Interpreting PCR Reports	63	56.76	75	67.57	0.01
Interpreting Molecular Detection of AMR Gene	31	27.93	35	31.53	0.31
Microbiological Findings Requiring Antimicrobial Treatment	51	45.95	84	75.68	0.00
Interpretation of Rapid Diagnostic Tests	68	61.26	72	64.86	0.31
Differentiating Colonizers, Contaminants, from True Pathogens	37	33.33	43	38.74	0.06
Correct Drug-Bug Combination in AST Report	67	60.36	76	68.47	0.05
Average	47	43.06	69.6	62.70	0.00
Theme 4: Antimicrobial Stewardship (AMS) Actions					
Optimizing Duration of Antibiotic Treatment	89	80.18	96	86.49	0.07
Best Practices for Indwelling Urinary Catheter Use in Hospitalized Patients	107	96.40	105	94.59	0.31
Effective infection control measures during an outbreak in a healthcare setting	91	81.98	91	81.98	0.58
Avoiding Antimicrobial Misuse in Acute Diarrhea	56	50.45	87	78.38	0.00
Antibiotic de-escalation based on AST	46	41.44	83	74.77	0.00
Timely Initiation of Antimicrobial Therapy in Sepsis	46	41.44	83	74.77	0.00
Evidence-Based Approach to Viral Infections	41	36.94	61	54.95	0.03
Identifying Double Redundant Antibiotic Cover	40	36.04	47	42.34	0.56
Avoid Overtreatment of Asymptomatic Bacteriuria	73	65.77	82	73.87	0.05
Review and Timely De-escalation	85	76.58	79	71.17	0.19
Average	67	60.72	81	73.33	0.01
Theme 5: AMS Strategies					
Concept of Diagnostic Stewardship	24	21.62	45	40.54	0.00
Role of Multidisciplinary AMS Committee	105	94.59	106	95.50	0.50
Surgical prophylaxis best practices	98	88.29	94	84.68	0.19
Knowledge of Antibiotic Policy	89	80.18	97	87.39	0.50
Utilizing Hospital Antibigrams for Informed Antibiotic Selection	80	72.07	82	73.87	0.14
Selective Reporting of Antimicrobial Susceptibility Testing as a strategy to promote appropriate antibiotic use	53	47.75	78	70.27	0.03
Monitoring High Usage and Reserve Antibiotics	63	56.76	76	68.47	0.01
Audits and Feedback to Prescribers to promote responsible antibiotic prescribing.	64	57.66	91	81.98	0.02
AWaRe Categorization of Antibiotics	53	47.75	82	73.87	0.01
Formulary Restriction Strategies	43	38.74	78	70.27	0.00
Average	67	60.54	83	74.68	0.01

Discussion:

Diagnostic uncertainty significantly contributes to antibiotic overuse. Effective diagnostic stewardship aims to reduce unnecessary testing while ensuring essential diagnostics, guided by sound clinical judgment^{10,18}. Instead of broad syndromic testing or delaying treatment for exhaustive investigations, diagnostic stewardship promotes judicious, timely diagnostics based on clinical and epidemiological context. In this study, only 21% of participants initially understood this concept, with many misinterpreting diagnostic stewardship as multiplex testing for all pathogens. Post-intervention understanding rose to 40.5%, a modest but significant improvement, highlighting persistent misconceptions and the need for continued targeted education.

Participants demonstrated significant improvement in pre-analytical practices for blood culture: recognition of optimal timing rose from 45% to 59%, correct blood culture collection techniques (including number of sets, volume per bottle, and draw order) from 49% to 67.6%, and awareness of proper transport conditions rose from 63% to 83%. However, understanding of appropriate indications for blood cultures improved only slightly (33% to 38%). Blood cultures yield positive results in only 5–30% of cases, often due to inappropriate testing and poor practices^{19–22}. The intervention emphasized reserving blood cultures for patients with high pre-test probability (e.g., fever, hypotension, central venous catheters) rather than routine ICU protocols, as unnecessary testing leads to false positives and increased antimicrobial use^{10,22}. Clinical decision tools such as SIRS, SOFA, NEWS, and Shapiro criteria were highlighted to guide rational test ordering and timely empiric therapy^{23,24}.

Hospitalized patients often face increased diagnostic burden, higher costs, and a greater risk of AMR^{25–26}. This often stems from initiating antibiotics upon detecting any organism, regardless of true infection versus colonization. Rational urine culture use requires clear indication, proper collection, and interpretation in clinical context. Post-intervention in the present study, recognition of appropriate indications improved markedly (45% to 75%), and participants better understood that pyuria or cloudy urine especially in catheterized patients does not justify culturing in asymptomatic patients, as these often reflect

inflammation or colonization rather than infection^{25–26}. Baseline knowledge of proper urine specimen collection from indwelling catheters was high (97%), consistent with IDSA guidelines by Hooton et al.²⁷. However, post-intervention, this declined slightly to 93%, possibly due to overgeneralization of guidance on catheter replacement whereas recommended it is only for long-term catheterization (>2 weeks) when infection is suspected, not routinely for specimen collection²⁷. This underscores the need to clarify distinctions between routine collection practices and specific recommendations in training content.

Understanding of urine culture interpretation improved significantly. Recognition of clinically significant bacteriuria in symptomatic patients rose from 41% to 77% and participants correctly identified that only pure growth of uropathogens at $\geq 10^5$ CFU/mL warrants treatment, while mixed growth or lower counts often indicate contamination. Awareness about unnecessary antibiotics in asymptomatic bacteriuria also improved (65% to 73%), with more participants recognizing that, except in pregnancy, urological procedures, or immunocompromised states, asymptomatic patients do not require antibiotics as also reported in²⁸.

Antimicrobial overuse and inappropriate microbiological testing remain major challenges in respiratory tract infections (RTIs) especially in outpatient setting. Most upper RTIs are viral and clinically diagnosed, yet antibiotics are frequently overprescribed^{29,30}. Screening tools and scoring systems such as the Modified CENTOR score help clinicians differentiate viral from bacterial infections and guide rational antibiotic prescribing³¹. These structured approaches minimize unnecessary cultures and antibiotic use, supporting evidence-based care while reducing AMR risk. Incorporating such scoring systems into routine outpatient workflows strengthens stewardship by promoting targeted testing and therapy rather than empirical treatment. Correct responses on antibiotic use in pharyngitis improved from 36.9% to 54.9%, indicating better clinical judgment. However, lower RTIs showed minimal improvement: understanding of respiratory culture indications rose only from 25% to 32%, and differentiation between colonization and infection from 33% to 38%. False-positive cultures often lead to overtreatment, while negative results may cause premature

discontinuation. For example, *Candida* in sputum of immunocompetent patients usually indicates colonization, not infection³². These modest gains highlight the need for focused, case-based training on microbiological data interpretation.

Awareness of evidence supporting shorter antibiotic courses for stable, uncomplicated community acquired pneumonia (CAP) improved slightly (80% to 86%) post-intervention, reinforcing existing knowledge. Current guidelines and randomized controlled trials recommend limiting therapy in CAP to 5 days in clinically stable patients, as shorter regimens are non-inferior to longer courses and reduce adverse effects, *C. difficile* risk, and antimicrobial resistance³³.

Antibiotic misuse in watery diarrhea is another concern, as most cases are viral and self-limiting, requiring only supportive care³⁴. Before the intervention, 50% of participants recognized that antibiotics are unnecessary; this improved to 78% post-intervention, indicating a positive shift in awareness³⁴. In hospital setting, inappropriate or excessive testing for *C. difficile* can misattribute colonization as infection, leading to unnecessary antibiotics and inflated hospital-acquired infection rates³⁵. Before the intervention, only 25% of participants identified correct indications; this improved modestly to 32% post-intervention. Testing should be reserved for patients with recent antibiotic exposure, ≥ 3 unformed stools in 24 hours, and no recent laxative use, while "tests of cure" should be avoided³⁵.

Most respondents (82%) correctly identified the importance of hand hygiene and isolation protocols during outbreak scenarios, both pre- and post-intervention, indicating strong theoretical understanding. However, incorrect responses such as initiating empirical antibiotics or dismissing stool culture value, highlight the need for ongoing education to reinforce evidence-based practices. Consistent integration of infection control training into routine clinical practice is essential to minimize transmission and safeguard patient and staff safety.

Correct identification of acceptable samples (e.g., pus aspirated in a syringe) improved from 63% to 86%, while errors such as choosing catheter tips, formalin-fixed tissue, or superficial swabs persisted, underscoring the need for continued training³⁶.

Selecting appropriate antimicrobials based on culture and susceptibility reports is central to DS,

requiring consideration of spectrum, drug-pathogen match, and pharmacokinetic /pharmacodynamic (PK/PD) parameters³⁷. Post-intervention, correct selection using BMQ (breakpoint/MIC quotient) improved significantly (35% to 69%), but recognition of redundant antibiotic coverage showed only modest gains (36% to 42%), such as avoiding unnecessary combinations like metronidazole with meropenem³⁷. Understanding of selective and cascade reporting as AMS strategies improved from 47.7% to 70.2% in the present study. These approaches encourage narrow-spectrum antibiotic use and provide alternative options based on outcomes or additional diagnostics, reserving broad-spectrum agents for complex cases^{38,39}.

Interpretation of serological markers improved significantly (22.5% to 57.7%), especially for CRP and procalcitonin. Pre-intervention, many participants assumed elevated biomarkers alone justified starting antimicrobials. The intervention clarified that these markers may be low in early infection or high in non-infectious conditions and are better suited for guiding de-escalation or cessation rather than initiation, consistent with prior findings⁴⁰.

Post-intervention, correct interpretation of rapid diagnostic tests (RDTs) improved slightly (61.3% to 64.9%), indicating modest gains in understanding key principles such as sensitivity, specificity, and the need for confirmatory ELISA for accurate diagnosis in dengue. This aligns with national dengue management guidelines, which emphasize that while NS1-based RDTs offer early detection, their variable sensitivity and specificity necessitate confirmatory ELISA to avoid false results and inappropriate treatment decisions⁴¹. Also, proper awareness of proper timing for serological tests remained low, with Widal test knowledge increasing only from 25% to 32%, despite well-documented limitations of rapid tests (like *Typhidot®* and the Widal test) and high misdiagnosis risk^{42–44}. Given the test's low accuracy and high risk of misdiagnosis, there is a need for continued education and transitioning to more reliable diagnostic methods^{43,44}. While RDTs are convenient and widely used, clinicians must recognize that their performance varies by sensitivity and specificity, and results should be interpreted cautiously particularly for conditions like dengue, and enteric fever.

Molecular diagnostics enhance detection of AMR genes and reduce turnaround time but cannot distinguish colonization from infection^{45,46}. Their increasing use such as multiplex PCR panels and rapid molecular assays for bloodstream infections has improved early pathogen identification and resistance gene detection, supporting timely clinical decisions. However, these technologies have limitations that gene presence doesn't always indicate phenotypic resistance and absence doesn't ensure susceptibility⁴⁷. Gene presence does not always indicate phenotypic resistance, nor does absence guarantee susceptibility⁴⁷. This understanding was low and improved slightly (28% to 32%), highlights a critical gap in clinical practice, their misinterpretation can lead to inappropriate antibiotic choices, either overtreatment or undertreatment. To address this, emphasis is needed that molecular results are not to be used in isolation; they must be integrated with culture-based methods and clinical context for accurate interpretation.

Understanding of AMU and need for AMR monitoring improved markedly (63% to 96%). Awareness of audits for tracking antibiotic use, especially high-priority and reserve drugs, rose from 58% to 82%. Knowledge of WHO's AWaRe classification increased from 48% to 74%, emphasizing prioritization of Access antibiotics to preserve Watch and Reserve groups and guide formulary decisions.

Awareness of DS varied widely, influenced by specialty and experience. Microbiologists, senior prescribers, and clinicians with >5 years' experience had higher baseline knowledge. In high workload settings, time and resource constraints, risk aversion, and diagnostic uncertainty drive antibiotic overuse⁴⁸. These behavioral drivers combined with limited confidence in interpreting complex diagnostics underscore the need for structured education and decision-support tools to reduce reliance on defensive prescribing and promote evidence-based testing.

Gaps in knowledge among doctors can be addressed during undergraduate and postgraduate training. Although AMS was introduced in the undergraduate curriculum 2019, less than 0.6% of competencies focus on AMR and AMS⁴⁹. Major broad specialties also lack AMS emphasis, and DS is absent from most curricula, leaving students undertrained in pre-analytical processes and

diagnostic interpretation. Infectious disease clinicians currently bridge this gap, but their numbers are limited. The National Medical Commission's antimicrobial prescriber module 2024 introduced in 2024 is a positive step, though its full impact will emerge gradually as new graduates adopt evidence-based practices⁵⁰.

Implementing DS faces significant barriers, especially in ICUs where clinicians often avoid delaying or de-escalating antimicrobials in complex cases^{5,12}. While reducing unnecessary testing DS can curb overtreatment, but it risks delayed diagnosis in high-risk patients. A balanced approach, introduced during induction and in-service training, is essential to optimize testing strategies. Given curriculum gaps and evolving diagnostics, ongoing multidisciplinary training integrating infection prevention & control, antimicrobial and diagnostic stewardship is the solution to bridge theory-practice gaps and ensure rational antibiotic use.

Study strengths and limitations:

The online format enabled participation from diverse specialties nationwide, ensuring efficiency and inclusiveness. Of 458 enrolled clinicians, 111 completed all pre- and post-tests; attrition was likely due to scheduling conflicts and the five-day requirement. Despite this, the varied backgrounds of completers support representativeness. Strategies like chat support and interactive case sessions helped engagement. Virtual platforms show promise as scalable, cost-effective tools for nationwide capacity-building. However, assessing long-term impact on clinical practice requires future longitudinal studies.

Conclusion

Diagnostic and antimicrobial stewardship are complementary strategies essential for optimizing patient care. Because diagnostic testing strongly influences antibiotic prescribing, strengthening clinicians' diagnostic competencies is important. Our educational intervention improved foundational competency to promote evidence-based use of appropriate test selection, interpretation of culture and molecular reports, and evidence-based use of rapid diagnostic tests in clinical decision-making. However, persistent gaps in some areas such as misconceptions about

serological tests, overreliance on molecular diagnostics without phenotypic confirmation highlight the need for ongoing, targeted training and structured follow-up to sustain behavioral change. Integrating AMS and DS into all medical curricula and decision-support tools, will help sustain behavioral change and promote rational antimicrobial use in the long term, while interim structured programs for early-career and practicing clinicians remain vital to address the evolving challenge of AMR.

Declarations:

Human Ethics and Consent to Participate: The study complied with ethical standards by maintaining participant confidentiality and ensuring voluntary participation. Since the study used anonymous, non-identified data and focused on educational improvement with no risk to participants, it was exempt from ethical review in accordance with the ICMR Guidelines for Ethical Review for Human Participants, 2017 by Institutional Ethics Committee of Institute of Human Behavior and Allied Sciences, Delhi, India⁵¹.

Availability of data and materials: The analyzed data is available in the supplementary information files set whereas the raw data or any other data generated during the current study will be made available from the corresponding author on reasonable request.

Competing interests:

None.

Authors contributions:

SS contributed to the study's conception, design development & execution and data collection. RG contributed to development & execution of the modules, pre- and post-assessments and performed the data analysis. SS and RG jointly prepared the first draft of the training modules and manuscript. AW, SR, VM, KKC, EG and SD contributed to the development of the modules and provided critical feedback on draft manuscript. All authors reviewed and approved the final manuscript.

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Conflict of interest:

None.

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