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Original Research Article

Enhancing microbiological culture accuracy: clinical audit on the role of direct microscopy and the Bartlett scoring system in diagnostic correlation of sputum samples

Preethika Ravi, Morubagal Raghavendra Rao, Tejashree A., Vidyavathi B. Chitharagi*

Department of Microbiology, JSS Medical College, Mysuru, Karnataka, India

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***Correspondence:**
Dr. Vidyavathi B. Chitharagi,
E-mail: vidyavathi@jssuni.edu.in

ABSTRACT

Background: Lower respiratory tract infections (LRTIs) represent a significant clinical burden necessitating accurate microbiological diagnosis to guide targeted antimicrobial therapy. The quality of sputum specimens substantially influences the validity of culture results and diagnostic reliability. The Bartlett scoring system (BS) is a standardized, objective tool for assessing sputum specimen quality. However, its clinical utility in predicting culture positivity within tertiary care settings remains inadequately characterized in existing literature.
Methods: A clinical audit was conducted on 212 consecutive sputum samples from patients with suspected LRTIs collected during September and October 2023 at a tertiary care center. All specimens were subjected direct microscopy examination with concurrent assessment using the Bartlett scoring system. Samples that met quality criteria (BS≥2) were processed for culture using standard microbiological techniques. Culture positivity rates were correlated with Bartlett scores to determine the association between sample quality assessment and microbiological yield.
Results: Among the 212 sputum samples analyzed, specimens with higher Bartlett scores demonstrated significantly increased culture positivity rates, establishing a positive correlation between sample quality assessment and microbiological diagnostic yield. Samples classified as high-quality (BS≥2) demonstrated superior diagnostic utility compared to lower-quality specimens. The Bartlett scoring system, when applied systematically in conjunction with direct microscopy, facilitated objective specimen selection and enhanced laboratory processing efficiency.
Conclusions: This audit demonstrates that the scientific utility of Bartlett scoring system serves as a valuable objective tool for assessing sputum quality and predicting culture positivity.

Keywords: Sputum culture, Clinical audit, Sputum smear microscopy, Lower respiratory tract infection, Bartlett scoring system

INTRODUCTION

Lower respiratory tract infections (LRTIs) remain a leading cause of global morbidity and mortality, necessitating precise identification of causative pathogens for effective individualized therapy and containment of antimicrobial resistance.[1-3] The microbiological diagnosis of LRTIs hinges on the quality of the sputum specimen. High-quality samples increase the likelihood of isolating true respiratory pathogens, whereas samples contaminated by oral flora can misguide diagnosis and promote inappropriate antimicrobial use.[4,5] International guidelines from the American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) emphasize specimen quality as an indispensable component of the diagnostic workflow for pneumonia and LRTIs.[6,7] They recommend

that sputum samples should be obtained by deep coughing, ideally producing grossly purulent material, and explicitly call for cytological screening under microscopy before bacteriological analysis.[8]

The Bartlett scoring system is widely validated as an international standard for microscopic quality assessment of sputum.[9-12] It stratifies samples based on the count of neutrophils (polymorphonuclear leukocytes) and squamous epithelial cells (SECs), reliably differentiating between specimens originating from lower respiratory tract infection and those contaminated by upper respiratory tract secretions.[13,14,30] Acceptable samples typically demonstrate >25 neutrophils and <10 SECs per low-power field, correlating with enhanced pathogen yield and clinical relevance.[15-17] Incorporating such quality control measures substantially increases the detection rate of clinically meaningful pathogens and reduces misdiagnosis due to colonizing flora, as corroborated by multi-center studies and systematic reviews.[18-21] Furthermore, stringent quality assessment and rejection of poor-quality samples not only optimize laboratory resources but also foster rational antibiotic prescribing, thereby curbing the emergence and spread of antimicrobial resistance.[22-25]

This clinical audit evaluates the diagnostic performance of the Bartlett scoring system, correlates culture results to BS grades, and contextualizes findings within current international standards for microbiological quality assurance and stewardship.[6,9,18] By rigorously applying the Bartlett criteria and integrating global recommendations, this study advances the reliability and actionable value of sputum culture diagnostics for LRTIs, supporting both clinical decision-making and antimicrobial stewardship in diverse healthcare settings.

METHODS

Study design and sample collection

A retrospective analysis was conducted on 212 sputum samples obtained consecutively in JSS Hospital, Mysore during September and October 2023 from patients presenting with clinical or radiological suspicion of lower respiratory tract infection (LRTI). Patients were instructed to provide sputum samples following established protocols to optimize specimen quality. The best time for collection was early morning, after rinsing the mouth with water, to minimize contamination by oral flora. Each sample was required a minimum of 3-5 ml of purulent or mucoid material, collected in a sterile, labelled container, and transported to the laboratory within 2-4 hours or refrigerated if a delay was anticipated.

Patients presenting with clinical signs (productive cough, dyspnea, chest discomfort) or radiological evidence (infiltrates, consolidation on imaging) of lower respiratory tract infection have been included in the study. Amongst these samples that have been collected following standardized protocols have been included in the study.

Sample processing

Quality assessment and direct microscopy

Purulent portions of each sputum sample was selected for evaluation. Slides were prepared for direct Gram staining and microscopy. Sputum quality was determined using the Bartlett scoring system, which assesses the presence of mucus, number of polymorphonuclear leukocytes (PMNs), and squamous epithelial cells (SECs) per low-power field (LPF).

Bartlett's scoring system

Presence of mucus: +1; number of PMNs and SECs; +2 points: >25 PMNs and <10 SECs per LPF; +1 point: 10-25 PMNs and <10 SECs per LPF; 0 points: <10 PMNs and <10 SECs or >10 SECs per LPF; -1 or -2 points: >10 SECs per LPF with few/no PMNs, indicating salivary contamination.

Each slide was systematically examined under oil immersion, counting cells in at least 20-30 LPFs to ensure accuracy and minimize sampling bias.

Specimen rejection criteria

Samples with a Bartlett score ≤0 were interpreted as contaminated or non-acceptable, as per consensus guidelines. These samples were rejected for routine culture, and clinicians were advised to obtain repeat or alternate specimens in cases of persistent clinical suspicion.

Microbiological analysis

All specimens were subjected to direct microscopy prior to culture. Only samples meeting acceptability criteria (Bartlett score ≥1) proceeded to culture. Standard aerobic and anaerobic culture techniques were performed on all accepted samples, following the latest Clinical Laboratory Standards Institute (CLSI) recommendations for specimen plating, incubation, and pathogen isolation. Bacteria and fungi were identified using conventional biochemical and chromogenic methods, supplemented by automated systems where available.

Antimicrobial susceptibility testing

Isolates of clinical relevance was further processed for antimicrobial susceptibility testing as per CLSI guidelines, using manual and automated phenotypic methods.

Data analysis

Culture positivity, microbial yield, and pathogen spectrum were compared across Bartlett score categories (+2, +1, 0, -1, -2). Rates of specimen rejection and contamination were recorded. The diagnostic value of the Bartlett scoring system was evaluated by correlating culture isolation rates

with microscopic quality strata, using descriptive statistics and chi-square analyses wherever appropriate.

RESULTS

Out of the 212 samples that were analysed, BS score (30) of +1 and a score of BS -0 were of similar numbers i.e. 67 (31.6 %), 61 (28.7%) had a score of BS +2, 9 (4.24%) had a score of BS -1, 6 (2.8%) had a score of BS -2. Among the BS+2 category 15 (7%) yielded growth of respiratory pathogens and based on AST pattern, the antibiotic therapy was started. In the BS+1 category 4 (1.9%) yielded growth of pathogens. In the BS-0, 9 (4.24%) yielded the growth of pathogens, *Acinetobater baumanii* growth was noted in 1 (0.4%) sample with BS -1. Among the pathogens, *Klebsiella pneumoniae* 13 (6.1%) was the most commonly isolated pathogen followed by Acinetobacter baumannii 4 (2%), *Pseudomonas aeruginosa* 3 (1.41%), MRSA (1%) *Hemophilus influenzae* and *Aspergillus* spp. was grown in one sample each (0.4% each). Among the BS-0 samples, 6 samples had clinical manifestations of LRTI (lower respiratory tract infection) in view of that the samples were processed inspite of the low BS score. Among 2 samples with BS +2, with suggestive clinical manifestation did not yield growth. Similar finding was noted in 2 samples with BS +1 (Table 1).

Table 1: Bartlett scoring of sputum samples and corresponding culture interpretation.

Bartlett score	Month	Growth	Organism	No growth
+1	September (n=30)	2	*Kleb.pneumoniae*-1, *Ps.aeruginosa*-1	28
	October (n=37)	2	*Enterobacter* spp-1, *Acinetobacter* complex-1	35
+2	September (n=37)	5	*Kleb.pneumoniae*-3, MRSA-1, *Aspergillus* spp-1	32
	October (n=24)	10	*Ps.aeruginosa*-1, *Kleb.pneumoniae*-3, MRSA-1, Alpha hemolytic Strep-1, *E.coli*-1, *H.influenzae*-1, *Enterobacter cloacae*-1, *Acinetobacter* complex-1	14
0	September (n=33)	6	*Kleb.pneumoniae*-3, MDR *Kleb.pneumoniae*-1, *Ps.aeruginosa*-1, *Aspergillus* spp-1	27
	October (n=36)	3	*Kleb.pneumoniae*-2, *Acinetobacter* complex-1	33
-1	September (n=4)	0	-	4
	October (n=5)	1	*Acinetobacter* complex-1	4
-2	September (n=3)	0	-	3
	October (n=3)	0	-	3

Table 2: Key organism isolated by BS score.

Bartlett score	Main organisms isolated	Culture negative samples	Total samples	Culture positivity [%]
2	*Klebsiella pneumoniae*, MRSA, *Aspergillus*	46	61	24.6
1	*Klebsiella pneumoniae*, *P. aeruginosa*, *Acinetobacter*	63	67	6
0	MDR *Klebsiella pneumoniae*, *P. aeruginosa*	60	69	13
-1, -2	*Acinetobacter* [rare]	14	15	6.7

Most frequent isolates: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (including MRSA), *Acinetobacter* spp., *Enterobacter* spp., *Aspergillus* spp. Highest yield and diversity of pathogens were observed in samples graded +2, supporting data from prior studies. Contaminated samples (BS≤0) produced scanty growth, often limited to commensal or colonizer organisms (Table 2).

DISCUSSION

As per the recommendation samples showing score of BS-0 or less should be rejected and asked for repeat sample when clinically indicated.[30] In this Audit 38.6% of the samples with score of 0 or less were processed. However, 6 (8.9%) samples which yielded the pathogens had a clinical significance indicating the importance of processing samples with BS-0. A total of 91.1% of the samples were processed without any clinical significance which accounted for wastage of resources. As per guidelines samples needs to be processed for culture before doing microscopic examination. So this affects the rejection of samples for culture before microscopy. Other factor to be considered is the technical error while preparation of smears which can affect the Bartlett score reporting. The samples with BS score 2+ (22%) helped in the maximum identification and a guide for antibiotic therapy and good clinical outcomes. But one finding in the study was the paradoxically less number of pathogens isolated from samples with BS+1 score (0.5%). Assessment by the Bartlett scoring system-using the ratio of neutrophils to squamous epithelial cells (SECs)-enables laboratories to identify high-quality samples that more likely contain causative organisms.[7,8] Numerous studies demonstrate that high Bartlett scores are strongly predictive of increased culture positivity rates and specificity for clinically relevant pathogens, including *Streptococcus pneumoniae*, *Haemophilus influenzae* and

Moraxella catarrhalis.[4,9,10] In contrast, low-quality samples often yield a predominance of commensals, non-pathogenic or multi-drug-resistant organisms, increasing the risk of microbiological misinterpretation and inappropriate therapy (Table 1 and 2).[4,11]

The accurate selection of sputum samples for microbiological analysis guided by standardized scoring system directly supports antimicrobial stewardship programs.[12,13] Excluding poor-quality specimens reduces unnecessary broad-spectrum antibiotic use, which is a principal driver of antimicrobial resistance globally.[8,14,15] Laboratories implementing routine quality assessment prior to culture report a decrease in indiscriminate antibiotic prescriptions, improved targeting of therapy, and a measurable reduction in the length of hospital stay and costs associated with respiratory infections.[8,15]

Rejected or low-quality sputum samples can yield results that confound clinical decision-making by producing non-specific or misleading microbial profiles.[4,10,16] This increases diagnostic uncertainty, delays optimal treatment, and potentially exposes patients to prolonged or inappropriate antibiotic courses.[9] On the other hand, mandatory routine scoring practices like Bartlett's system enhance actionable reporting, offering rapid, reliable guidance for clinicians in managing LRTIs in line with international best practices.[5,6,8] Laboratory adoption of sputum quality assessment according to recognized guidelines not only improves the reliability of diagnostic outcomes but is also essential for resource optimization, patient safety, and stewardship of antimicrobial effectiveness.[12,15,17]

Limitations

The retrospective design prevents control of variables affecting specimen collection and documentation, introducing information bias regarding clinical and radiological criteria. Cross-contamination with oral flora despite standardized collection protocols remains difficult to assess retrospectively and may have resulted in false identification of commensal organisms as pathogens.

CONCLUSION

The Bartlett scoring system plays a crucial role in improving the diagnostic evaluation of sputum samples from patients with lower respiratory tract infections (LRTIs). Since sputum is often contaminated with oral flora, accurate interpretation can be difficult, leading to misleading clinical conclusions. By quantitatively assessing neutrophils, squamous epithelial cells, and mucus, the Bartlett method distinguishes representative lower respiratory samples from those with contamination or poor cellular content. Scores of zero or below generally indicate unsuitable samples for culture, conserving laboratory resources, although the audit noted that a minority of such samples still yielded significant pathogens, highlighting the importance of repeat collection when infection is strongly suspected. In contrast, specimens scoring two or above were more likely to yield clinically relevant bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*, as well as nosocomial organisms like *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. These findings affirm the system's value as an effective triage tool, enabling precise microbial identification and informed antimicrobial therapy selection.

While the Bartlett method facilitates antimicrobial stewardship by reducing unnecessary cultures and limiting the overuse of broad-spectrum antibiotics, challenges remain in its application. Current guidelines recommending culture prior to microscopic quality assessment introduce logistical barriers for rejecting poor-quality samples, while variability in smear preparation and interpretation can affect scoring accuracy. Additionally, the retrospective, single-center design and limited sample size of this audit suggest the need for larger, prospective multicenter studies to validate these findings across diverse populations.

Nevertheless, the Bartlett scoring system stands out as a scientifically sound and practical framework for enhancing sputum culture diagnostics, optimizing laboratory resources, and supporting better patient care and public health outcomes

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