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Comparison of Two Different Criteria for Judging the Acceptability of Sputum Specimens

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Abstract: ***Introduction: Sputum Gram stains and cultures are standard tests for the diagnosis and management of lower respiratory tract infections. Collection of sputum sample, microscopy and culture plays important role. Aim: Aim of this study was to analyze the diagnostic performance of Gram staining by Bartlett and Murray and Wshington ceiteria comparison to sputum culture results for lower respiratory tract infections. Materials and methods: This Study was performed in department of Microbiology in a tertiary care hospital. The study period was one year from January 2023 to December 2023. Gram staining and culture were done for all 173-sputum sample. The evaluation of expectorated sputum based on Bartlett, Murray and Washington grading system. Results: Among 173 samples, 150 (86.7%) samples accepted and 23 (13.29%) were unacceptable by Bartlett whereas Murray and Washington accepted 109 samples. The percentage of specimen found acceptable which contained potential growth varied from 82.63% (Bartlett) to 80.7% (Murray and Washington). The sensitivity, specificity of Bartlett criteria are 97.6%, %, 43.4%. whereas the sensitivity, specificity of M &W criteria are 77%, 64%. Among culture positive Klebsiella pneumonia were most common 39 (36.11%), followed by E. coli 23 (21.29%%), Pseudomonas aeruginosa 21 (19.4%) Staphylococcus aureus 15 (13.8%%), Acinatobacter baumanii 5 (4.6%) and Serratia marcessens 2 (1.8%) Streptococcus pneumonia 3 (2.7%). Conclusion: Correct interpretation of Gram stain helps the physician to start the antibiotics early and can improve the choice of antibiotics thus can greatly reduce morbidity and mortality in critically ill patients***

Keywords: Bartlett' s and Murray & washington grading system, Gram stain, Sputum acceptable category, Sputum culture

1. Introduction

The most common infectious disease with the highest mortality and morbidity rates globally are the LRTIs (Lower respiratory tract infections). To diagnose the LRTIs (lower respiratory tract infections) in the microbiological laboratory, the most frequently employed method is the microscopic examination of expectorated sputum samples[1]. Sputum culture takes much more time to give results as compared to Gram stain, that's why Gram stain is valuable in guiding empirical treatment for the patient[2]. However, the clinical usefulness of sputum cultures and Gram stains is questioned due to possible contamination of oropharyngeal normal flora and the complexity of etiological pathogen. Several criteria have been proposed to evaluate the quality of sputum samples. These criteria have different combinations and cutoffs for the minimum number of squamous epithelial cells (SEC) and polymorphonuclear leukocytes (PMN) per low - power field[3 - 4]. In 1974, Bartlett first suggested that clinical laboratories should examine sputum specimens microscopically and refuse to culture specimens showing evidence of excessive oropharyngeal contamination[5]. Using this system, negative numbers are assigned to a smear when squamous epithelial cells are observed, indicating contamination with oropharyngeal secretions (saliva). Positive numbers are assigned for the presence of segmented neutrophils, indicating the presence of active inflammation. The magnitude of these negative and positive determinations depends on the relative numbers of epithelial cells and segmented neutrophils. A final score of 0 or less indicates either lack of inflammatory response or presence of significant salivary contamination, thus invalidating the specimen. In 1975, Murray and Washington described a simpler scheme for judging the quality of sputum specimens. The large number of epithelial cells in groups 1 to 4 of this system indicates contamination with oropharyngeal secretions and invalidates the samples (i. e., the specimen should be rejected). Only group 5 specimens are considered clinically relevant[5 - 6].

2. Material and Method

This Study was performed in department of Microbiology in a tertiary care hospital. The study period was one year from January 2023 to December 2023. Gram staining and culture were done for all 173 sputum sample. The evaluation of expectorated sputum based on Bartlett, Murray an d Washington grading system. Each specimen was then categorized as accepted or rejected by each of the two different criteria (Table 1.) Smear was prepared for Gram staining from the purulent portion of sputum. Stained smear was examined microscopically under low power and oil immersion. Low power magnification was used to detect and quantitate squamous epithelial cells and neutrophills; however, microorganisms were observed under oil immersion. The most purulent portion of each specime n was inoculated onto blood, chocolate, and MacConkey agars. Then samples were streaked out by a medical technician using a standard 4 - quadrant streaking method and incubated at 35°C with 5% CO2 for 48 hours.

Table 1: Grading System for Assessing the Quality of Sputum Samples

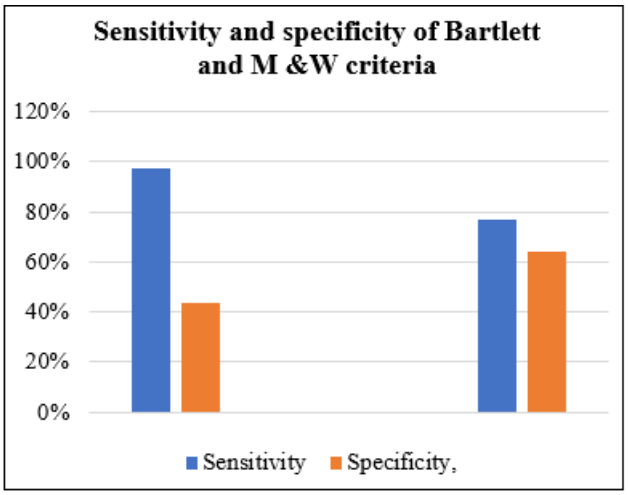
Bartlett				Murray and Washington		
No. of Neutrophils per 10× Low - Power Field	Grade	No. of Epithelial Cells per 10× Low - Power Field	Grade	Grade	No of Epithelial Cells per Low - Power Field	No. of Neutrophils per 10 Low - Power Field
<10	0	10–25	- 1	Group 1	10	25
10–25	+1	>25	- 2	Group 2	10–25	25
>25	+2			Group 3	25	25
Presence of mucus	+1			Group 4	25	10–25
				Group 5	<10	25

3. Result

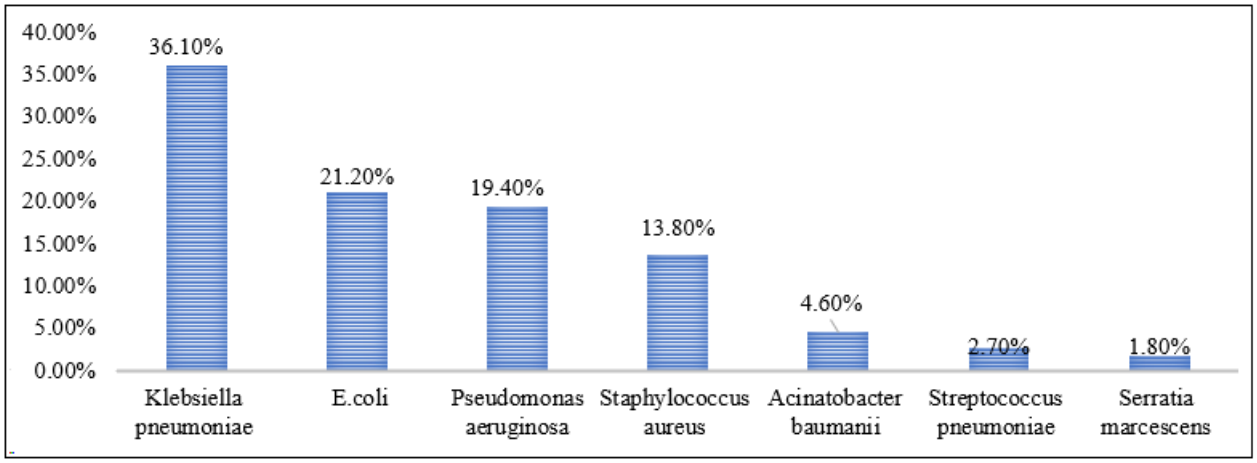
Out of 173 samples, 150 (86.7%) samples accepted and 23 (13.29%) were unacceptable by Bartlett where as Murray and Washington accepted 109 samples as shown table no.2. The difference in the number of specimens rejected by these two criteria was statistically significant (P < 0.01, chi - square test). The percentage of specimen found acceptable which contained potential growth varied from 82.63% (Bartlett) to 80.7% (Murray and Washington). The sensitivity, specificity of Bartlett criteria are 97.6%, %, 43.4%. where as The sensitivity, specificity of M &W criteria are 77%, 64% as shown graph no.1. . Among culture positive *Klebsiella pneumonia* were most common *39 (*36.11%), followed by *E. coli 23 (21.29%%), Pseudomonas aeruginosa 21 (*19.4%) *Staphylococcus aureus 15 (13.8%%), Acinatobacter baumanii 5 (4.6%)* and *Serratia marcessens 2 (1.8%) Streptococcus pneumonia 3 (2.7%*) as shown graph no.2.

Table 2: Microscopic examination of 173 expectorated sputum specimens, applying two different criteria for acceptance or rejection

Method	No. of sample accepted	No. of sample rejected	No. of potential growth (accepted sample)	No. of potential growth (rejected sample)
Bartlett	150	23	124 (82.6%)	3 (13%)
Murray and Washington	109	64	88 (80.7%)	26 (40.6%)



Graph 1: Sensitivity and Specificity of two different criteria



Graph 2: Potential growth of pathogens in sputum sample

4. Discussion

An expectorated sputum specimen which contains a potential pathogen presents a diagnostic dilemma to the physician[7]. It is often impossible to determine whether the potential pathogen is an etiological agent or represents oropharyngeal contamination. The amount of oropharyngeal contamination can be judged by evaluating the relative number of squamous epithelial cells in the specimens. Those specimens that are obviously contaminated are less likely to yield interpretable results, although potential pathogens may be present. Without microscopy, culture results are of unknown relevance and results may be misleading. Hence diagnosing respiratory infection by sputum culture without microscopic examination invites confusion and misinformation. To minimise the effect of oropharyngael contamination on lower respiratory tract secretions, Bartlett, Murray and Washington devised screening criteria based on quantitation of leucocytes and squamous epithelial cells [8]. The use of gram stained smears to assess the quality of sputum samples has received considerable attention as a means for improving the reliability of sputum culture.9. In our study 86.7% sputum sample were accepted by Bartlett' s criteria which is similar to study done by wong et al[7], who found 83% sample accepted by using Bartlett grading system. In the present study among unacceptable sample by Bartlett criteria 13% revealed potential pathogen growth which is similar to 9.5% isolation rate reported by Mariraj J. et al[8.] In our study 36.9% sputum samples were unacceptable by Murray and Washington criteria whereas M&W reported 45% rejection in their study. For assessment of the microorganisms isolated from this study it was seen that the most common isolated organism was isolated *Klebsiella pneumonia* was most common *39 (*36.11%), followed by *E. coli 23 (21.29%%), Pseudomonas aeruginosa 21 (*19.4%) *Staphylococcus aureus 15 (13.8%%), Acinatobacter baumanii 5 (4.6%)* and *Serratia marcessens 2 (1.8%) Streptococcus pneumonia 3 (2.7%).* This result is similar to Renu Goel el al[1]. In 2022 and Ziyade N et al.[10] in 2010 where authors reported similar microorganisms to be isolated from the expectorated sputum. Question is remaining which method is most reliable to be determine. in our study potential pathogen in unacceptable sample was less in Bartlett criteria as compare to Murray and Washington.

5. Conclusion

Our experience shows that a clinician should accept a microbiologist's judgment that a specimen heavily contaminated with saliva should not be cultured as sputum. The microbiology laboratory must use objective Gram stain screening by Bartlett' s criteria before inoculation into culture media. Hence the routine sputum Gram stain is essential to provide meaningful culture report and it helps clinician to start imperical treatment thus can greatly reduce morbidity and mortality.

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