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Sputum Gram Stain Assessment in Relation to Sputum Culture for Respiratory Tract Infections in A Tertiary Care Hospital

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ABSTRACT

For diagnosis of Lower respiratory tract infections (LRTIs), expectorated sputum is the most commonly received sample in the laboratory. Normal resident bacteria of the oropharynx contaminate the sputum sample. The value of sputum microscopy and culture in the diagnosis of LRTIs is a matter of controversy. The following study was conducted to evaluate the correlation of Gram's stain and culture in sputum samples from LRTIs. A total of 50 sputum samples were processed. All the samples were processed through Gram's stain and culture. The Gram's smear was examined for the presence of polymorphs, epithelial cells and bacterial forms. Sputum samples were evaluated based on Bartlett's grading system. The cultures were identified by standard protocols and antibiotic susceptibility performed by Kirby Bauer disk diffusion method. Out of 50 sputum samples processed, 21 (42%) were acceptable and 29 (58%) were not meeting the standard criteria of Bartlett. Potential pathogens were obtained from 17 of 21 acceptable samples (80.95 %), and from11 of 29 non-acceptable samples (27.58%). The most common organisms isolated in the present study include: Klebsiella pneumoniae (22%) and Pseudomonas aeruginosa (8%). we recommend initial screening of sputum samples for clinically relevant results, and reject the non-acceptable samples, and re-order for fresh and correct specimens.

KEYWORDS: Sputum, Gram's stain, Bartlett's criteria, sputum culture.

Introduction

Lower respiratory tract infections (LRTIs) are a common cause of morbidity and mortality worldwide. For diagnosis of LRTIs, expectorated sputum is the most commonly received sample in the laboratory, which can be obtained easily and non-invasively. Normal resident bacteria of the oropharynx usually contaminate the sputum sample, and a large number of different species overgrow preventing the determination of the true pathogen.[1]Another commonly seen problem is that unless the patients are well trained, most of the times it is watery saliva which is sent instead of the purulent sputum to the laboratory, leading to erroneous results. The value of sputum microscopy and culture in the diagnosis, management and outcome of LRTIs is a matter of controversy. Traditional recommendations for management usually include Gram's stain and culture of sputum for routine evaluation. Some proponents feel that a properly performed sputum Gram's stain examined according to the correct guidelines is definitely useful in the initial evaluation of patients with pneumonia. Some others suggest that sputum Gram's stain and culture are neither sensitive nor specific for diagnosis of Community Acquired Pneumonia (CAP).[2]With the above set back ground, the following study was conducted to evaluate the correlation of Gram's stain and culture in sputum samples from LRTIs

Materials and methods:

The present study was done from April to September 2013 after obtaining Institutional Ethical Committee Approval. A total of 50 sputum samples were processed during the study period. All the 50 sputum samples were processed through Gram's stain and inoculation onto Blood agar, Nutrient agar and MacConkey agar. The Gram's smear was examined under low power, oil immersion and observed for the presence of polymorphs, epithelial cells and bacterial forms. Sputum samples were evaluated based on Bartlett's grading system and a score was given.

Table – 1 - Barlett criteria

Number of Neutrophils /10 X LPF	GRADE
<10	0
10-25	+1
>25	+2
Presence of mucus	+1
Number of Epithelial Cells /10 X LPF	
10-25	-1
>25	-2

Average number of epithelial cells and neutrophils in 20-30 LPFs was calculated and the total score was arrived. A final score of 0 or less indicated lack of active inflammation or contamination (non-acceptable sample), and a score of 1 and above was considered an acceptable sample. A Gram's stain was done and the culture was made. The organisms were identified by standard protocols and antibiotic susceptibility of recommended drugs was performed by Kirby Bauer disc diffusion method.

Results

Based on Bartlett's screening criteria, out of 50 sputum samples processed, 21 (42%) were acceptable and 29 (58%) were not meeting the standard criteria. Potential pathogens were obtained from 28 of 50 sputum samples , of which 17 are from acceptable samples (60.71%), and 11 are from non-acceptable samples (39.28%).[Table-2]

Table - 2 - Results

ORGANISM	NO	(%)
Klebsiella pneumoniae	11	22
Pseudomonas aeruginosa	4	8
Non fermentor GNB	3	6
Candida	2	4
Proteus mirabilis	2	4
Staphylococcus aureus	2	4
MR cons	1	2
Escherichia coli	1	2
Acinetobacter	1	2
Commensal of oral cavity	1	2

The organisms obtained from the non-acceptable category (11 of 29) included K. pneumonia (5), Pseudomonas aeruginosa (2) ,MR CoNS, Candida, E. coli, Non fermentor GNB 1 each.

Discussion

Examination of expectorated sputum has been the primary means of determining the causes of bacterial pneumonia. However, lower respiratory tract secretions are always contaminated with upper tract flora present in the saliva. Hence, sputum is among the least clinically relevant specimens received for culture in the Microbiology laboratory, even though it is one of the most numerous and time consuming specimens. Grading system for sputum does not apply for lower respiratory tract infections by Legionella species, Mycobacterium tuberculosis, fungi and virus. The importance of micro-organisms recovered from respiratory samples must always be evaluated in light of clinical information [3]. Good sputum samples depend on thorough healthcare worker education and patient understanding [4].The sputum grading system was initially given by Bartlett. This gives an indication whether the specimen represents the site of infection [5].

Out of 50 sputum samples processed during two month period in the present study, 21 samples (42%) were considered in the acceptable category based on Bartlett's screening criteria used, and 29 samples (58%) in the non acceptable category. Other studies reporting similar percentages include Anevlavis et al- 63% and Mariraj et al- 79%. [5,6] While Daniel Musher et al had reported a low percentage of 31% acceptability,[7] Ravichandran et al had reported that all 74 (100%) of their sputum samples so screened were in the non acceptable category, which contained only mixed flora not acceptable for culture.[2]

Total culture positivity in the present study was 56% (28/50). Culture positivity reported in other studies include- Nihan Ziyade et al- 44.7%,Daniel Musher et al- 79%, Jean J Lloveras- 57%, Nawfal Ali Mubarak- 41.7%, Aroma Oberoi et al- 32% and Somporn et al- 40.95%. [1,7-11] On the contrary Ravichandran et al grew potential pathogens only in 5% of their specimens processed.[2]

Out of the 21 acceptable specimens in the present study, potential pathogens were grown in 17 samples (80.95%). While M R Shariatzadeh et al grew potential pathogens only

in 33.7% of their acceptable samples,[12] Mariraj et al grew in 63.2% of their acceptable samples.[6]

Among the 29 samples in the non acceptable category in the present study, pathogens were grown in only in 11 samples (37.93%). Mariraj et al [6] had reported 2 out of their 21 non acceptable samples had growth of potential pathogens (9.5%).

A useful quality assurance tool for sputum cultures is the comparison of Gram's stain and culture. If organisms seen in smear do not grow in culture, or if organisms that grow in moderate to heavy quantities are not seen in the smear, the smear should be re-evaluated. Gram's stain is a relatively in-sensitive method, so small numbers of bacteria in culture may not well be visualized in the smear.

The most common organisms isolated in the present study include: Klebsiella pneumoniae- 22%, Pseudomonas aeruginosa-8%, which correlates well with other studies. [1, 6, 8, 9, 10]

In a study by Ravichandran et al, the authors had concluded that sputum Gram's stain, sputum culture and blood cultures, in non severe CAP do not provide any diagnostically useful information and do not help in guiding for initial therapy. They concluded that the tests may be reserved for severe cases of CAP.[2]

In a study by Mariraj et al, the authors had concluded that Microbiology laboratories may reject for culture, those sputum samples which fail to meet the criteria of Bartlett for purulence, and sputum cultures must be ordered judiciously for documented episodes of LRTIs to provide a meaningful output. As authors of the present study, we completely agree with the conclusions of Mariraj et al, to reject such sputum samples which do not satisfy the Bartlett's criteria and order for fresh and proper samples.[6]

Conclusion

Criteria should be established by each laboratory for rejection of unsuitable sputum specimens. Although general guidelines exist, director of each laboratory must decide which parameters to utilize. When a specimen is rejected, the person who submitted it should be made aware and the rejection criteria should be clearly listed in the laboratory service area. Clinical relevance should be applied in Diagnostic Microbiology. The point is to provide clinicians with information that will allow them to give best care to their patients.

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