Correlation of Sputum Gram Stain and Sputum Culture for Respiratory Tract Infections in a Tertiary Care Hospital, Ballari, India

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A B S T R A C T

One of the most important uses of the Gram stain is to evaluate the quality of expectorated sputum received for routine bacteriological culture. An acceptable sample yields less than 10 squamous epithelial cells per low power field. The simplest and least expensive sample for the diagnosis of lower respiratory infections is expectorated sputum. The utility of this approach is the subject of controversy, as the sample is contaminated by oropharyngeal flora as it passes through the mouth. To evaluate the importance of sputum microscopy and its correlation with culture in sputum samples from LRTIs and its antibiotic sensitivity pattern, 1019 samples were taken for the study. Each stained smear was examined microscopically, and the cellular components were evaluated. Those samples which satisfied Bartlett’s criteria were processed by standard protocols. According to Bartlett’s screening criteria 552 (54%) were accepted and 467 (46%) samples were rejected from 1019 samples. Potential pathogens were recovered from 450 (81.6%) samples out of 552 accepted samples. The most common organism isolated was Streptococcus pyogenes, followed by Klebsiella spp., followed by Pseudomonas spp., Staphylococcus aureus, Escherichia coli, Citrobacter koseri, Enterobacter spp. Most of the isolates were found to be susceptible to Amikacin, followed by Gentamycin, and most of the isolates were resistant to Ampicillin.

Keywords
Correlation, Culture, Respiratory Infections.

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. One of the most important uses of the Gram stain is to evaluate the quality of expectorated sputum received for routine bacteriological culture. An acceptable sample yields less than 10 squamous epithelial cells per low power field.

The simplest and least expensive sample for the diagnosis of lower respiratory infections is expectorated sputum.

Objective

To evaluate the importance of sputum microscopy and its correlation with culture in sputum samples from Lower Respiratory
Tract Infections (LRTIs) and its antibiotic sensitivity pattern.

**Materials and Methods**

The study was done from January 2016 to June 2016 at Central microbiology laboratory, VIMS, Ballari. 1019 samples were taken for the study.

Each stained smear was examined microscopically under low power and oil immersion, and the cellular components were evaluated. The samples were inoculated onto Blood agar and Macconkey agar and incubated at 37˚C for 24 hrs.

Depending on the type of growth, various biochemical tests were performed to identify the pathogen.

*In vitro* Antimicrobial susceptibility testing was done according to CLSI guidelines, by the standard agar disc-diffusion method (Kirby-Bauer) on Mueller Hinton agar.

**Results and Discussion**

According to Bartlett’s screening criteria 552 (54%) were accepted and 467 (46%) samples were rejected from 1019 samples. Potential pathogens were recovered from 450 (81.6%) samples out of 552 accepted samples (Fig. 1).

The most common organism isolated was *Streptococcus pyogenes*, followed by *Klebsiella spp.*, followed by *Pseudomonas spp.*, *Staphylococcus aureus*, *Escherichia coli*, *Citrobacter koseri*, *Enterobacter spp.* Most of the isolates were found to be susceptible to Amikacin, followed by Gentamycin, and most of the isolates were resistant to Ampicillin. 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.

When potential pathogen is isolated from the sputum sample, it is often difficult to decide 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 samples.

To minimise the effect of oropharyngeal contamination on lower respiratory tract secretions among cultures, Bartlett, Murray and Washington devised screening criteria based on quantitation of leucocytes and squamous epithelial cells.

Bartlett proposed that purity of sputum samples be rated according to the relative concentration of polymorph nuclear neutrophils, squamous epithelial cells and mucus in gram stained smears.

Average the number of neutrophils and epithelial cells in about 20-30 different 10x microscopic fields and calculate the total. A final score of 0 or less indicates lack of active inflammation or contamination with saliva. Repeat sputum sample should be requested. 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.
Fig. 1 Number of samples accepted and rejected

|                | Accepted samples | Rejected samples |
|----------------|------------------|------------------|
|                | 46%              | 54%              |

| Potential pathogens | Non-pathogenic | 82% |
|---------------------|---------------|------|
|                     | 18%           |      |

|            | S. pyogenes | Klebsiella spp. | Pseudomonas spp. |
|------------|-------------|-----------------|------------------|
| AMIKACIN   | 57%         | 62%             | 58%              |
| GENTAMYCIN | 31%         | 40%             | 43%              |
| CIPROFLOXACIN | 55%       | 31%             | 22%              |
In a study conducted by Anuradaha Mokkapati et al., 65% samples were acceptable and 35% were not meeting the standard criteria. Potential pathogens were recovered from 89.74% acceptable samples, and 9.52% from non-acceptable samples. In a study conducted by Amudha et al., 42% were acceptable and 58% were not meeting the standard criteria. Potential pathogens were obtained from 60.71% acceptable samples and 39.28% from non-acceptable samples.

Culture positivity reported in other studies include- Jean J L loveras- 57%, Daniel Musher et al.,- 79%, Somponr et al.,- 40.95%, Nawfal Ali Mubarak- 41.7%, Aroma Oberoi et al.,- 32% and Nihan Ziyade et al.,- 44.7%. On the contrary Ravichandran et al., grew potential pathogens only in 5% of their specimens processed. 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. In the current study 552 (54%) were accepted and 467 (46%) samples were rejected from 1019 samples. Potential pathogens were recovered from 450 (81.6%) samples out of 552 accepted samples.

Each laboratory should establish criteria for rejection of sputum samples that are not suitable for culture. By applying the criteria, unnecessary sample processing can be avoided, technologist time and workload is reduced. The main aim is to achieve a clinical relevance in Diagnostic Laboratories and provide a meaningful culture report.

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